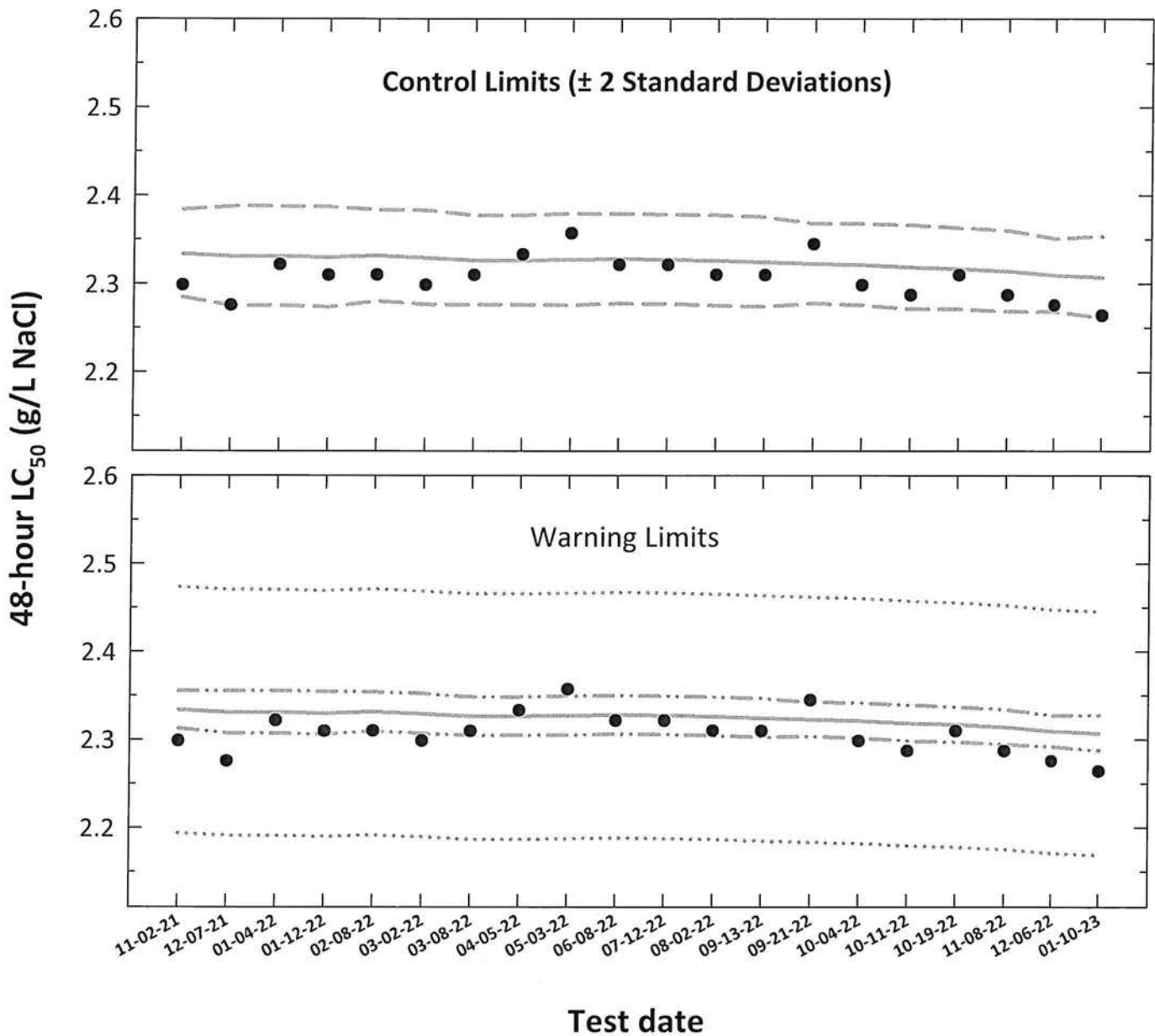


Ceriodaphnia dubia

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	11-02-21	2.2985	0.3614	0.3681	0.0046	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4758
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	06-08-22	2.3212	0.3657	0.3669	0.0047	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675
11	07-12-22	2.3212	0.3657	0.3668	0.0047	2.3272	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668
12	08-02-22	2.3099	0.3636	0.3666	0.0048	2.3260	2.2754	2.3779	2.3043	2.3483	2.1865	2.4656
13	09-13-22	2.3096	0.3635	0.3663	0.0048	2.3243	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637
14	09-21-22	2.3449	0.3701	0.3660	0.0042	2.3225	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619
15	10-04-22	2.2984	0.3614	0.3657	0.0043	2.3214	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607
16	10-11-22	2.2870	0.3593	0.3652	0.0044	2.3185	2.2715	2.3665	2.2982	2.3392	2.1794	2.4576
17	10-19-22	2.3096	0.3635	0.3649	0.0043	2.3168	2.2712	2.3633	2.2971	2.3368	2.1778	2.4558
18	11-08-22	2.2868	0.3592	0.3643	0.0043	2.3139	2.2685	2.3602	2.2943	2.3339	2.1750	2.4527
19	12-06-22	2.2755	0.3571	0.3635	0.0039	2.3093	2.2682	2.3510	2.2915	2.3274	2.1707	2.4478
20	01-10-23	2.2639	0.3549	0.3630	0.0043	2.3069	2.2613	2.3536	2.2871	2.3271	2.1685	2.4454

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

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

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

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

CV = Coefficient of variation.



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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaCIAC # 391

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 #: 2149

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	8.01	7.83	7.95
	Dissolved oxygen (mg/L)	7.7	7.0	7.0
	Conductivity (µmhos/cm)	300		
	Alkalinity (mg/L CaCO ₃)	63		
	Hardness (mg/L CaCO ₃)	86		
	Temperature (°C)	24.9	25.1	25.2
1750 mg/L	pH (S.U.)	7.99	7.88	7.91
	Dissolved oxygen (mg/L)	7.7	7.0	7.0
	Conductivity (µmhos/cm)	3410		
	Temperature (°C)	24.9	24.9	24.9
2000 mg/L	pH (S.U.)	8.00	7.92	7.95
	Dissolved oxygen (mg/L)	7.7	7.7	7.0
	Conductivity (µmhos/cm)	3920		
	Temperature (°C)	25.0	24.9	25.2
2250 mg/L	pH (S.U.)	8.00	7.93	7.99
	Dissolved oxygen (mg/L)	7.8	7.0	7.0
	Conductivity (µmhos/cm)	4200		
	Temperature (°C)	25.0	25.1	24.8
2500 mg/L	pH (S.U.)	7.99	7.92	8.01
	Dissolved oxygen (mg/L)	7.8	7.7	7.9
	Conductivity (µmhos/cm)	5090		
	Temperature (°C)	24.9	25.1	24.8
2750 mg/L	pH (S.U.)	8.00	7.93	8.02
	Dissolved oxygen (mg/L)	7.8	7.7	7.9
	Conductivity (µmhos/cm)	5310		
	Temperature (°C)	24.9	25.1	24.9

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	150664685

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 # 391

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-10-23	0530+ 0750	JL	0950	JL	283	BLUE	01-04-23B
24	01-11-23			0952	JL			
48 Termination	01-12-23			0950	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):	01-03-23 A+B
Age:	< 24-hours old
Date and time organisms were born between:	01-09-23 1435 TO 01-10-23 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.03 Temperature (°C): 25.0

Survival Data (number of living organisms):

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	3 ^{2d}	4 ^{1d}	4 ^{1d}	2 ^{3d}
48 Termination	2 ^{3d}	2 ^{3d}	1 ^{4d}	4 ^{1d}	0 ^{5d}	1 ^{4d}	1 ^{4d}	0 ^{5d}	0 ^{3d}	0 ^{4d}	0 ^{4d}	0 ^{2d}
Mean Survival	45%				10%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2195.2
Upper 95% confidence limit (mg NaCl/L)	2330.4
48-hour LC ₅₀ (mg NaCl/L)	2263.9

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date: 1/10/2023 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
 End Date: 1/12/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: CD-Ceriodaphnia dubia

Comments:

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

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.4500	0.4500	0.7351	0.4636	1.1071	36.604	4	10.00	10.00	11	20
*2500	0.1000	0.1000	0.3446	0.2255	0.4636	39.900	4	10.00	10.00	18	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

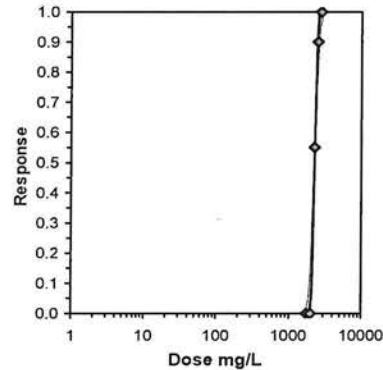
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77648	0.868	1.05649	5.29488
Equality of variance cannot be confirmed				

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

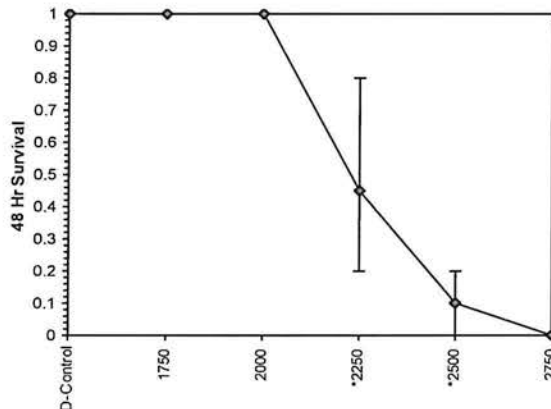
Treatments vs D-Control

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	35.7413	7.02955	21.9634	49.5192	0	1.71127	7.81472	0.63443	3.35486	0.02798	5
Intercept	-114.91	23.6004	-161.16	-68.65							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1948.8	1757.32	2047.17
EC05	3.355	2036.27	1883.52	2117.51
EC10	3.718	2084.49	1953.18	2157.4
EC15	3.964	2117.67	2000.75	2185.7
EC20	4.158	2144.41	2038.63	2209.27
EC25	4.326	2167.63	2070.97	2230.48
EC40	4.747	2227.24	2150.3	2289.56
EC50	5.000	2263.89	2195.15	2330.44
EC60	5.253	2301.15	2236.94	2376.29
EC75	5.674	2364.44	2299.64	2463.62
EC80	5.842	2390.03	2322.67	2501.68
EC85	6.036	2420.22	2348.58	2548.1
EC90	6.282	2458.74	2380.2	2609.27
EC95	6.645	2516.97	2425.83	2704.89
EC99	7.326	2629.93	2509.99	2898.21



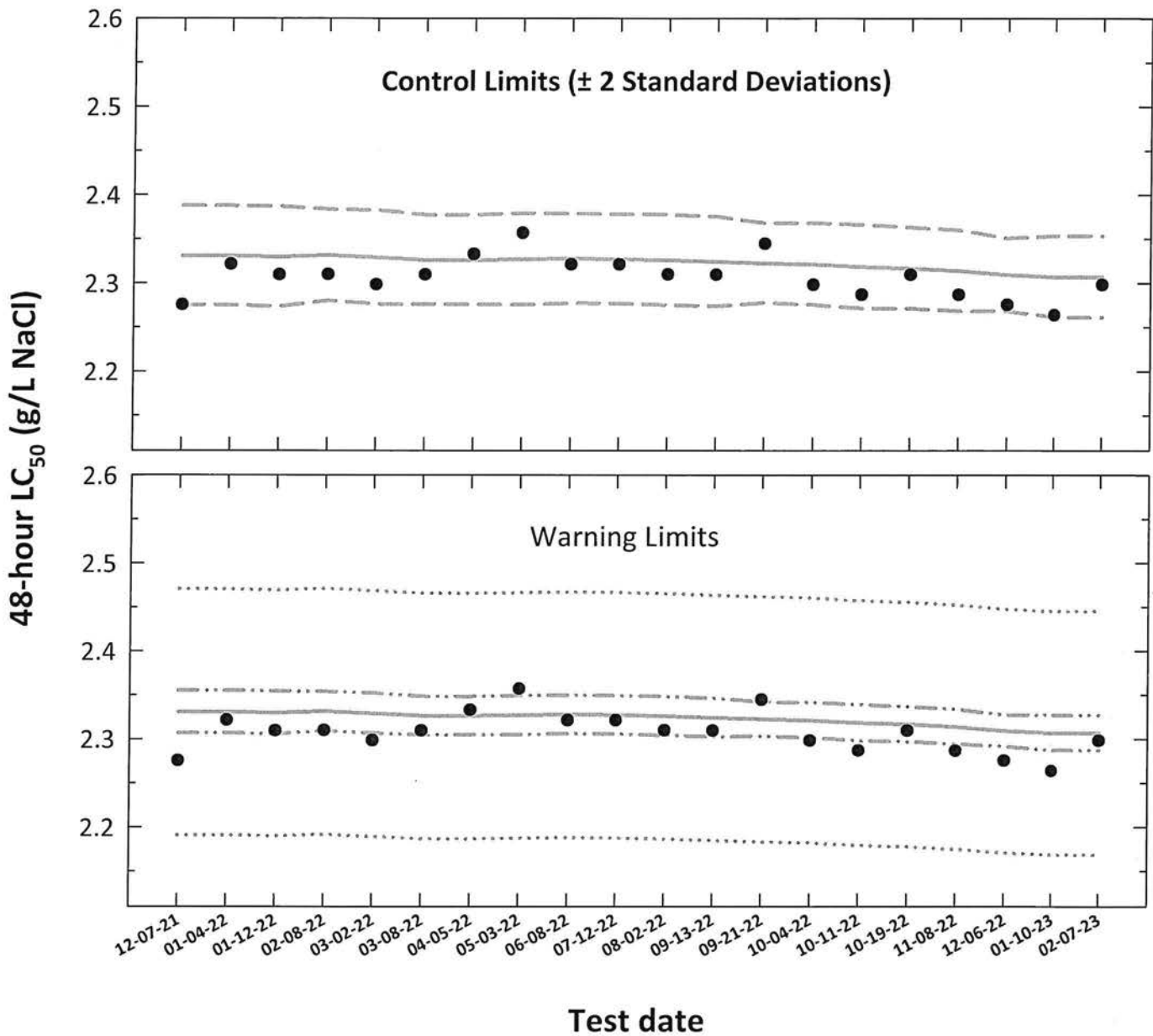
Dose-Response Plot



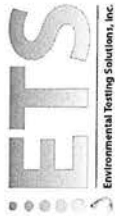
Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



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



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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L NaCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S		Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV		10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}	
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	06-08-22	2.3212	0.3657	0.3669	0.0047	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675
10	07-12-22	2.3212	0.3657	0.3668	0.0047	2.3272	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668
11	08-02-22	2.3099	0.3636	0.3666	0.0048	2.3260	2.2754	2.3779	2.3043	2.3483	2.1865	2.4656
12	09-13-22	2.3096	0.3635	0.3663	0.0048	2.3243	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637
13	09-21-22	2.3449	0.3701	0.3660	0.0042	2.3225	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619
14	10-04-22	2.2984	0.3614	0.3657	0.0043	2.3214	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607
15	10-11-22	2.2870	0.3593	0.3652	0.0044	2.3185	2.2715	2.3665	2.2982	2.3392	2.1794	2.4576
16	10-19-22	2.3096	0.3635	0.3649	0.0043	2.3168	2.2712	2.3633	2.2971	2.3368	2.1778	2.4558
17	11-08-22	2.2868	0.3592	0.3643	0.0043	2.3139	2.2685	2.3602	2.2943	2.3339	2.1750	2.4527
18	12-06-22	2.2755	0.3571	0.3635	0.0039	2.3093	2.2682	2.3510	2.2915	2.3274	2.1707	2.4478
19	01-10-23	2.2639	0.3549	0.3630	0.0043	2.3069	2.2613	2.3536	2.2871	2.3271	2.1685	2.4454
20	02-07-23	2.2984	0.3614	0.3630	0.0043	2.3069	2.2612	2.3535	2.2871	2.3271	2.1685	2.4454

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 # 392

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 #: 2167

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	<i>N</i>	<i>N</i>	<i>N</i>
Control, MHSW	pH (S.U.)	<i>7.30</i>	<i>7.49</i>	<i>7.37</i>
	Dissolved oxygen (mg/L)	<i>7.6</i>	<i>7.7</i>	<i>7.9</i>
	Conductivity (µmhos/cm)	<i>293</i>		
	Alkalinity (mg/L CaCO ₃)	<i>60</i>		
	Hardness (mg/L CaCO ₃)	<i>86</i>		
	Temperature (°C)	<i>24.9</i>	<i>25.2</i>	<i>25.0</i>
1750 mg/L	pH (S.U.)	<i>7.76</i>	<i>7.69</i>	<i>7.64</i>
	Dissolved oxygen (mg/L)	<i>7.8</i>	<i>7.8</i>	<i>7.8</i>
	Conductivity (µmhos/cm)	<i>3270</i>		
	Temperature (°C)	<i>24.9</i>	<i>24.9</i>	<i>24.9</i>
	2000 mg/L	pH (S.U.)	<i>7.78</i>	<i>7.68</i>
Dissolved oxygen (mg/L)		<i>7.8</i>	<i>7.9</i>	<i>7.7</i>
Conductivity (µmhos/cm)		<i>3730</i>		
Temperature (°C)		<i>25.0</i>	<i>24.9</i>	<i>24.9</i>
2250 mg/L	pH (S.U.)	<i>7.80</i>	<i>7.67</i>	<i>7.66</i>
	Dissolved oxygen (mg/L)	<i>7.8</i>	<i>7.9</i>	<i>7.7</i>
	Conductivity (µmhos/cm)	<i>4150</i>		
	Temperature (°C)	<i>25.0</i>	<i>25.1</i>	<i>25.1</i>
2500 mg/L	pH (S.U.)	<i>7.05</i>	<i>7.68</i>	<i>7.68</i>
	Dissolved oxygen (mg/L)	<i>7.8</i>	<i>8.0</i>	<i>7.7</i>
	Conductivity (µmhos/cm)	<i>4530</i>		
	Temperature (°C)	<i>25.0</i>	<i>25.0</i>	<i>24.9</i>
2750 mg/L	pH (S.U.)	<i>7.91</i>	<i>7.68</i>	<i>7.71</i>
	Dissolved oxygen (mg/L)	<i>7.8</i>	<i>8.1</i>	<i>7.8</i>
	Conductivity (µmhos/cm)	<i>5060</i>		
	Temperature (°C)	<i>25.0</i>	<i>25.2</i>	<i>24.9</i>

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	<i>130664685</i>



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 # 392

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	02-01-23	0520	JL	0158	JL	2B3	GREEN	02-01-23A
24	02-06-23			0155	JL			
48 Termination	02-09-23			0157	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):	01-31-23 A
Age:	< 24-hours old
Date and time organisms were born between:	02-06-23 1453 To 02-07-23 0520
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.66
	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	4 ^u d	4 ^u d	4 ^u d	4 ^u d
48 Termination	2 ^{3d}	3 ^{2d}	2 ^{3d}	3 ⁴	1 ^{4d}	1 ^{4d}	1 ^{4d}	1 ^{4d}	0 ^{4d}	0 ^{4d}	0 ^{4d}	0 ^{4d}
Mean Survival	50%				20%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

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

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date: 2/7/2023 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
End Date: 2/9/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: CD-Ceriodaphnia dubia
Comments:

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.4000	0.6000	0.4000	0.6000
2500	0.2000	0.2000	0.2000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

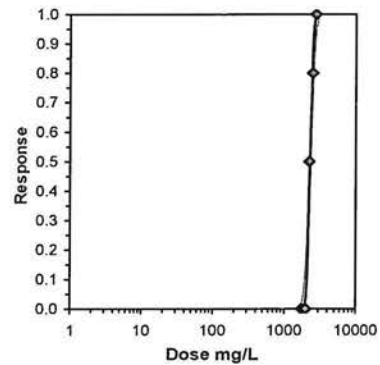
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5000	0.5000	0.7854	0.6847	0.8861	14.802	4	10.00	10.00	10	20
*2500	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	4	10.00	10.00	16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) Equality of variance cannot be confirmed	0.63123	0.868	2.7E-15	2.98039

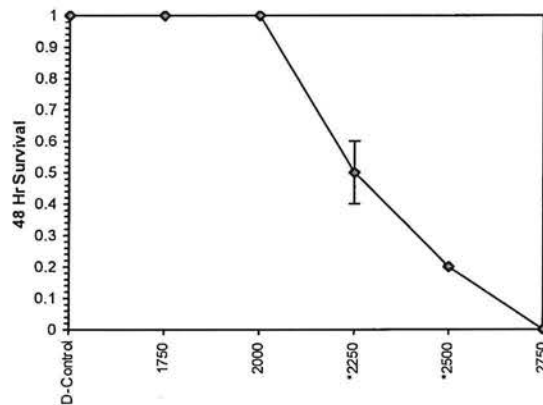
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test Treatments vs D-Control	2000	2250	2121.32	

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	30.6709	5.60227	19.6905	41.6514	0	2.7074	7.81472	0.43897	3.36142	0.0326	6
Intercept	-98.098	18.848	-135.04	-61.156							

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



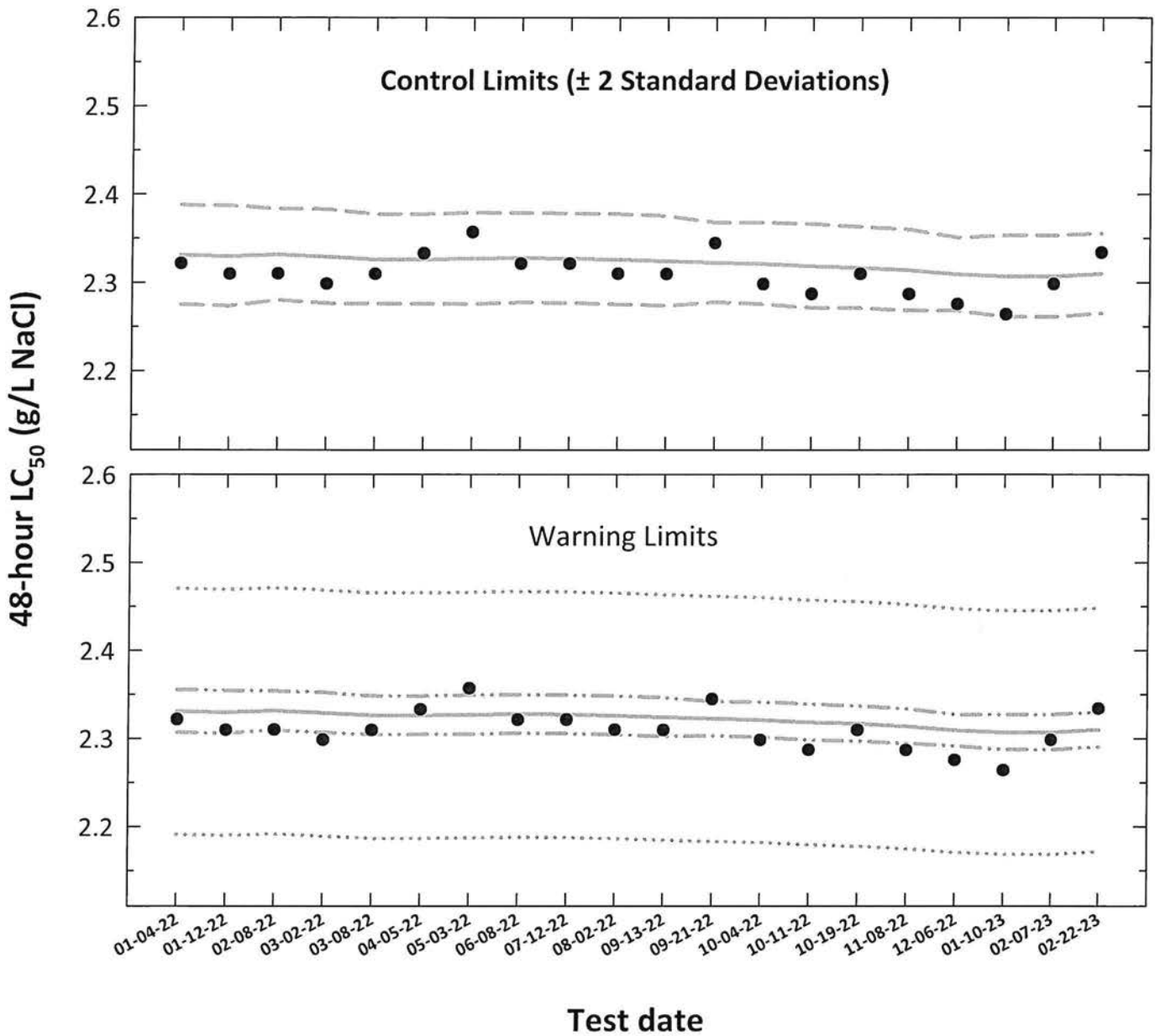
Dose-Response Plot



Ceriodaphnia dubia

Acute Reference Toxicant Control Chart

Source: In-house Culture



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}			
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	06-08-22	2.3212	0.3657	0.3669	0.0047	2.3278	2.2776	2.3791	2.3062	2.3498	2.1881	2.4675
9	07-12-22	2.3212	0.3657	0.3668	0.0047	2.3272	2.2770	2.3785	2.3056	2.3493	2.1876	2.4668
10	08-02-22	2.3099	0.3636	0.3666	0.0048	2.3260	2.2754	2.3785	2.3043	2.3483	2.1865	2.4656
11	09-13-22	2.3096	0.3635	0.3663	0.0048	2.3243	2.2739	2.3757	2.3026	2.3464	2.1848	2.4637
12	09-21-22	2.3449	0.3701	0.3660	0.0042	2.3225	2.2779	2.3681	2.3033	2.3421	2.1832	2.4619
13	10-04-22	2.2984	0.3614	0.3657	0.0043	2.3214	2.2755	2.3682	2.3016	2.3415	2.1821	2.4607
14	10-11-22	2.2870	0.3593	0.3652	0.0044	2.3185	2.2715	2.3665	2.2982	2.3392	2.1794	2.4576
15	10-19-22	2.3096	0.3635	0.3649	0.0043	2.3168	2.2712	2.3633	2.2971	2.3368	2.1778	2.4558
16	11-08-22	2.2868	0.3592	0.3643	0.0043	2.3139	2.2685	2.3602	2.2943	2.3339	2.1750	2.4527
17	12-06-22	2.2755	0.3571	0.3635	0.0039	2.3093	2.2682	2.3510	2.2915	2.3274	2.1707	2.4478
18	01-10-23	2.2639	0.3549	0.3630	0.0043	2.3069	2.2613	2.3536	2.2871	2.3271	2.1685	2.4454
19	02-07-23	2.2984	0.3614	0.3630	0.0043	2.3069	2.2612	2.3535	2.2871	2.3271	2.1685	2.4454
20	02-22-23	2.3339	0.3681	0.3636	0.0042	2.3099	2.2651	2.3555	2.2905	2.3296	2.1713	2.4485

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 # 393Dilution 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 #: 2167Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	7.93	7.98	8.10
	Dissolved oxygen (mg/L)	7.6	8.1	7.7
	Conductivity (µmhos/cm)	298		
	Alkalinity (mg/L CaCO ₃)	64		
	Hardness (mg/L CaCO ₃)	94		
	Temperature (°C)	24.8	25.1	25.2
1750 mg/L	pH (S.U.)	7.89	7.91	8.04
	Dissolved oxygen (mg/L)	7.6	8.0	7.7
	Conductivity (µmhos/cm)	3250		
	Temperature (°C)	24.9	24.9	25.0
2000 mg/L	pH (S.U.)	7.89	7.91	8.03
	Dissolved oxygen (mg/L)	7.6	8.1	7.7
	Conductivity (µmhos/cm)	3720		
	Temperature (°C)	24.9	25.2	25.0
2250 mg/L	pH (S.U.)	7.89	7.91	8.05
	Dissolved oxygen (mg/L)	7.7	8.1	7.7
	Conductivity (µmhos/cm)	4090		
	Temperature (°C)	24.8	25.2	24.9
2500 mg/L	pH (S.U.)	7.89	7.90	8.05
	Dissolved oxygen (mg/L)	7.8	8.1	7.6
	Conductivity (µmhos/cm)	4500		
	Temperature (°C)	25.0	25.0	24.9
2750 mg/L	pH (S.U.)	7.89	7.90	8.06
	Dissolved oxygen (mg/L)	7.8	8.1	7.7
	Conductivity (µmhos/cm)	4930		
	Temperature (°C)	25.0	25.0	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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaCIAC # 393

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	02-22-23	0600	JL	0802	JL	2B1	RED	02-21-23
24	02-23-23			0800	JL			
48 Termination	02-24-23			0800	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):	02-14-23 A4B
Age:	< 24-hours old
Date and time organisms were born between:	02-21-23 1500 TO 02-22-23 0600
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.46 Temperature (°C): 25.1

Survival Data (number of living organisms):

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	S	S	S	S	S	4 ^{1d}	4 ^{1d}	S	S	S	2 ^{3d}	3 ^{2d}
48 Termination	3 ^{2d}	4 ^{1d}	4 ^{1d}	3 ^{2d}	1 ^{4d}	1 ^{3d}	1 ^{3d}	0 ^{5d}	0 ^{5d}	0 ^{5d}	0 ^{1d}	0 ^{3d}
Mean Survival	70%				15%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2265.9
Upper 95% confidence limit (mg NaCl/L)	2399.8
48-hour LC ₅₀ (mg NaCl/L)	2333.9

Comments:

Test Reviewed by:

Acute Daphnid Test-48 Hr Survival

Start Date: 2/22/2023 Test ID: CdNaCIAC Sample ID: REF-Ref Toxicant
 End Date: 2/24/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: CD-Ceriodaphnia dubia

Comments:

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.6000	0.8000	0.8000	0.6000
2500	0.2000	0.2000	0.2000	0.0000
2750	0.0000	0.0000	0.0000	0.0000

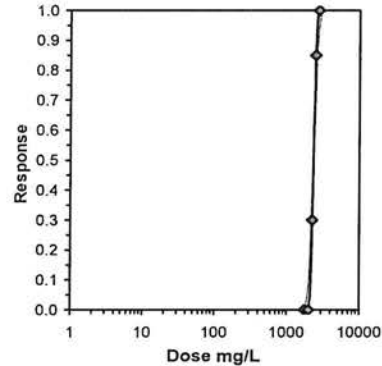
Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Number Resp	Total Number	
			Mean	Min	Max	CV%					
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4		0	20	
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.7000	0.7000	0.9966	0.8861	1.1071	12.807	4	10.00	10.00	6	20
*2500	0.1500	0.1500	0.4041	0.2255	0.4636	29.464	4	10.00	10.00	17	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.82099	0.868	-0.8875	1.61838

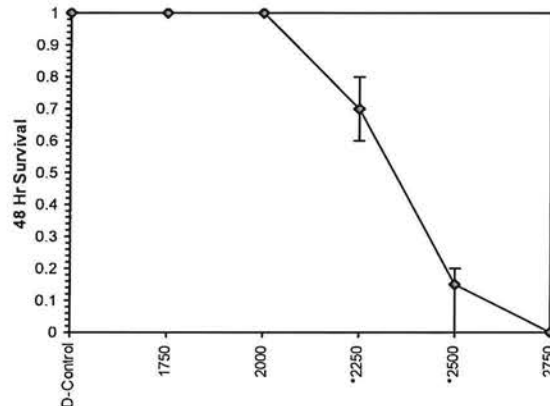
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	

Parameter	Value	SE	95% Fiducial Limits	Maximum Likelihood-Probit						
				Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	37.8059	7.63208	22.847 52.7648	0	0.32719	7.81472	0.95484	3.36809	0.02645	4
Intercept	-122.33	25.722	-172.75 -71.918							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	2025.59	1829.95	2123.56
EC05	3.355	2111.44	1956.14	2192.03
EC10	3.718	2158.68	2025.67	2230.83
EC15	3.964	2191.15	2073.07	2258.36
EC20	4.158	2217.3	2110.78	2281.31
EC25	4.326	2239.99	2142.95	2301.97
EC40	4.747	2298.19	2221.62	2359.69
EC50	5.000	2333.92	2265.88	2399.82
EC60	5.253	2370.21	2306.89	2444.99
EC75	5.674	2431.8	2368.01	2531.25
EC80	5.842	2456.68	2390.35	2568.86
EC85	6.036	2486	2415.45	2614.71
EC90	6.282	2523.39	2446	2675.09
EC95	6.645	2579.85	2490.03	2769.35
EC99	7.326	2689.18	2571.04	2959.49



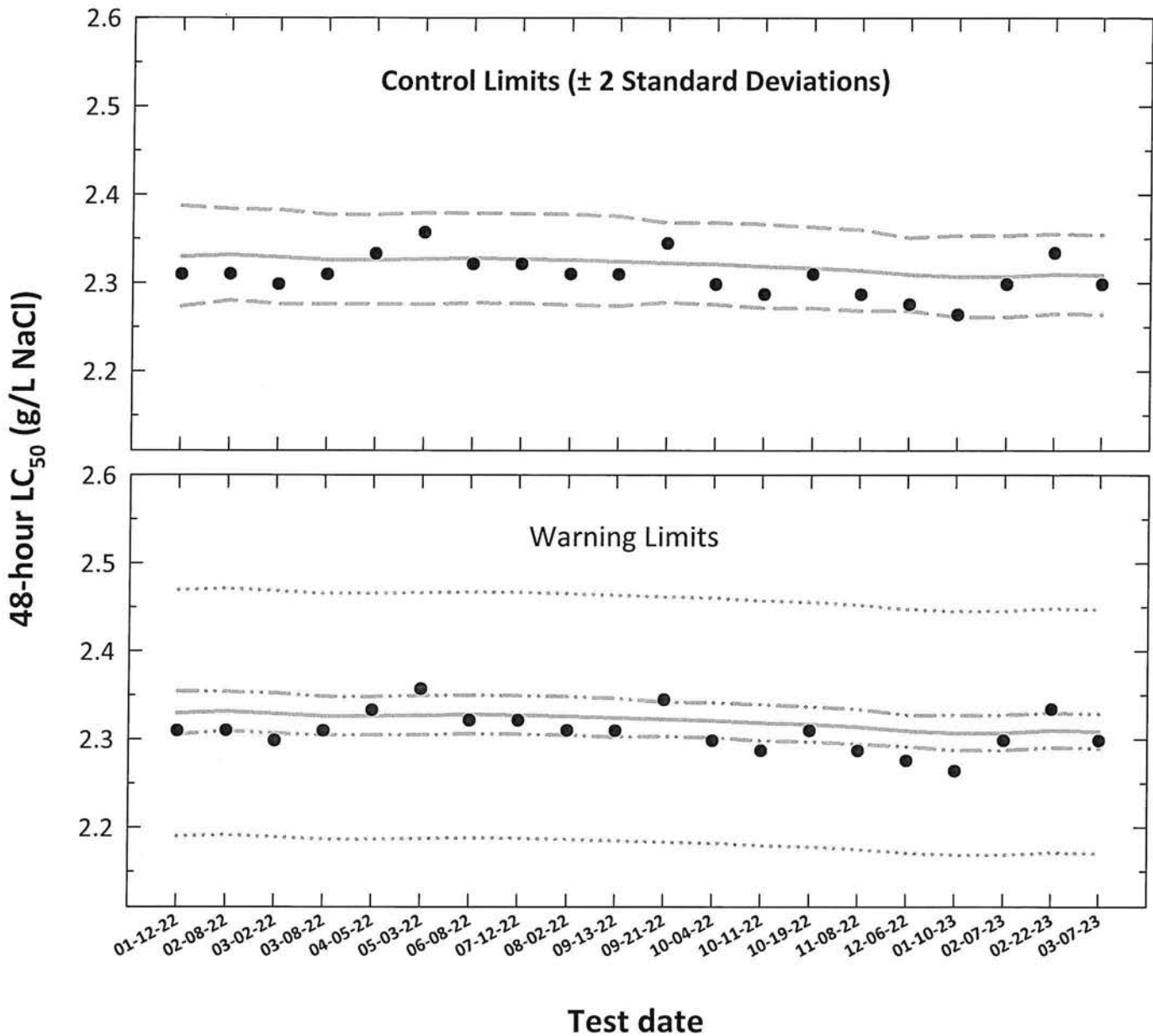
Dose-Response Plot



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 \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	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
2	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
3	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
4	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
5	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
6	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
7	06-08-22	2.3212	0.3657	0.3669	0.0047	2.3278	2.2776 2.3791	2.3062 2.3498	2.1881 2.4675
8	07-12-22	2.3212	0.3657	0.3668	0.0047	2.3272	2.2770 2.3785	2.3056 2.3493	2.1876 2.4668
9	08-02-22	2.3099	0.3636	0.3666	0.0048	2.3260	2.2754 2.3779	2.3043 2.3483	2.1865 2.4656
10	09-13-22	2.3096	0.3635	0.3663	0.0048	2.3243	2.2739 2.3757	2.3026 2.3464	2.1848 2.4637
11	09-21-22	2.3449	0.3701	0.3660	0.0042	2.3225	2.2779 2.3681	2.3033 2.3421	2.1832 2.4619
12	10-04-22	2.2984	0.3614	0.3657	0.0043	2.3214	2.2755 2.3682	2.3016 2.3415	2.1821 2.4607
13	10-11-22	2.2870	0.3593	0.3652	0.0044	2.3185	2.2715 2.3665	2.2982 2.3392	2.1794 2.4576
14	10-19-22	2.3096	0.3635	0.3649	0.0043	2.3168	2.2712 2.3633	2.2971 2.3368	2.1778 2.4558
15	11-08-22	2.2868	0.3592	0.3643	0.0043	2.3139	2.2685 2.3602	2.2943 2.3339	2.1750 2.4527
16	12-06-22	2.2755	0.3571	0.3635	0.0039	2.3093	2.2682 2.3510	2.2915 2.3274	2.1707 2.4478
17	01-10-23	2.2639	0.3549	0.3630	0.0043	2.3069	2.2613 2.3536	2.2871 2.3271	2.1685 2.4454
18	02-07-23	2.2984	0.3614	0.3630	0.0043	2.3069	2.2612 2.3535	2.2871 2.3271	2.1685 2.4454
19	02-22-23	2.3339	0.3681	0.3636	0.0042	2.3099	2.2651 2.3555	2.2905 2.3296	2.1713 2.4485
20	03-07-23	2.2984	0.3614	0.3634	0.0042	2.3087	2.2641 2.3542	2.2894 2.3284	2.1702 2.4472

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.

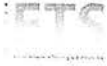
S = Standard deviation of the LC₅₀ values.

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 # 394

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 #: 2167

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	U	BLN	U
	Dissolved oxygen (mg/L)	8.16	8.03	8.04
	Conductivity (µmhos/cm)	7.8	7.8	8.0
	Alkalinity (mg/L CaCO ₃)	290		
	Hardness (mg/L CaCO ₃)	59		
	Temperature (°C)	92		
1750 mg/L	pH (S.U.)	25.0	25.2	24.9
	Dissolved oxygen (mg/L)	8.10	7.97	8.04
	Conductivity (µmhos/cm)	7.9	7.9	7.7
	Temperature (°C)	3250		
2000 mg/L	pH (S.U.)	25.1	25.2	25.1
	Dissolved oxygen (mg/L)	8.07	7.96	8.01
	Conductivity (µmhos/cm)	7.9	7.9	7.7
	Temperature (°C)	3760		
2250 mg/L	pH (S.U.)	25.1	25.0	25.1
	Dissolved oxygen (mg/L)	8.02	7.87	8.02
	Conductivity (µmhos/cm)	7.8	7.9	7.7
	Temperature (°C)	4160		
2500 mg/L	pH (S.U.)	24.9	24.9	25.0
	Dissolved oxygen (mg/L)	8.03	7.96	8.01
	Conductivity (µmhos/cm)	7.9	7.9	7.7
	Temperature (°C)	4600		
2750 mg/L	pH (S.U.)	24.9	25.1	25.2
	Dissolved oxygen (mg/L)	8.05	7.97	8.00
	Conductivity (µmhos/cm)	7.9	8.0	7.7
	Temperature (°C)	5060		

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

Chemical analyses:

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

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaCIAC # 394

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-01-23	0530	JL	0827	JL	2B2	Bwe	02-28-23A
24	03-06-23			0815	JL			
48 Termination	03-09-23			0822	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):	02-28-23 D
Age:	< 24-hours old
Date and time organisms were born between:	03-06-23 1445 TO 03-01-23 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.00
	Temperature (°C): 24.9

Survival Data (number of living organisms):

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

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

Comment codes: d = dead, u = unhealthy

Statistics:

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

Comments:



Acute Daphnid Test-48 Hr Survival

Start Date: 3/7/2023 Test ID: CdNaClAC Sample ID: REF-Ref Toxicant
 End Date: 3/9/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: CD-Ceriodaphnia dubia
 Comments:

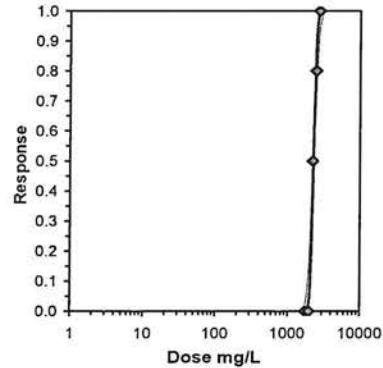
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.6000	0.2000	0.6000	0.6000
2500	0.0000	0.2000	0.4000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Number Resp	Total Number	
			Mean	Min	Max	CV%					
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4		0	20	
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5000	0.5000	0.7805	0.4636	0.8861	27.063	4	10.00	10.00	10	20
*2500	0.2000	0.2000	0.4594	0.2255	0.6847	40.823	4	10.00	10.00	16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

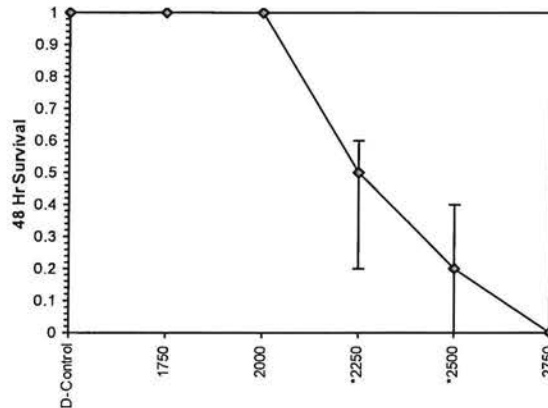
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) Equality of variance cannot be confirmed	0.72325	0.868	-1.225	3.75298

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

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	30.6709	5.60227	19.6905	41.6514	0	2.7074	7.81472	0.43897	3.36142	0.0326	6
Intercept	-98.098	18.848	-135.04	-61.156							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	1930.04	1733.59	2037.4							
EC05	3.355	2031.36	1873.25	2120.31							
EC10	3.718	2087.52	1950.89	2167.38							
EC15	3.964	2126.29	2004.15	2200.75							
EC20	4.158	2157.62	2046.72	2228.51							
EC25	4.326	2184.86	2083.19	2253.43							
EC40	4.747	2255.05	2173.24	2322.55							
EC50	5.000	2298.35	2224.66	2370.05							
EC60	5.253	2342.48	2273	2423.1							
EC75	5.674	2417.72	2346.49	2523.77							
EC80	5.842	2448.25	2373.78	2567.61							
EC85	6.036	2484.32	2404.68	2621.1							
EC90	6.282	2530.46	2442.6	2691.68							
EC95	6.645	2600.43	2497.7	2802.26							
EC99	7.326	2736.93	2600.25	3026.95							



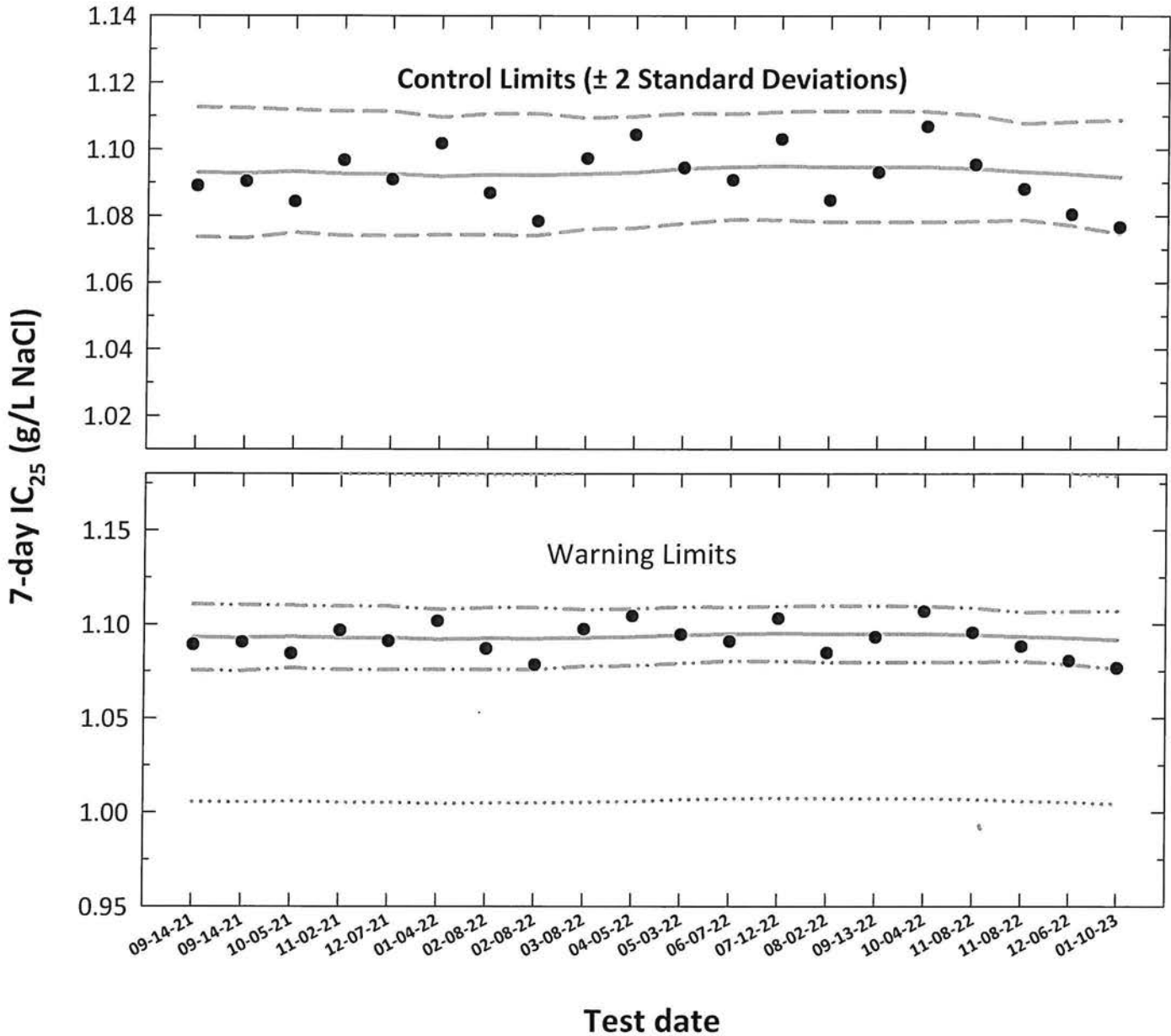
Dose-Response Plot



Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- · - · - **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- · · · · **USEPA Warning Limits** (mean logarithmic IC₂₅ ± S_{A,10} converted to anti-logarithmic values, S_{A,10} = 10th percentile of CVs reported nationally by USEPA)

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

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		10th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,10}	CT + S _{A,10}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	07-12-22	1.1030	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
14	08-02-22	1.0847	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
15	09-13-22	1.0931	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
16	10-04-22	1.1068	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823
17	11-08-22	1.0954	0.0396	0.0391	0.0032	1.0943	1.0785	1.1103	1.0798	1.1087	1.0067	1.1818
18	11-08-22	1.0881	0.0367	0.0387	0.0029	1.0932	1.0788	1.1078	1.0800	1.1064	1.0057	1.1807
19	12-06-22	1.0805	0.0336	0.0385	0.0031	1.0926	1.0772	1.1083	1.0785	1.1068	1.0052	1.1800
20	01-10-23	1.0767	0.0321	0.0381	0.0034	1.0916	1.0748	1.1088	1.0762	1.1071	1.0043	1.1790

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.

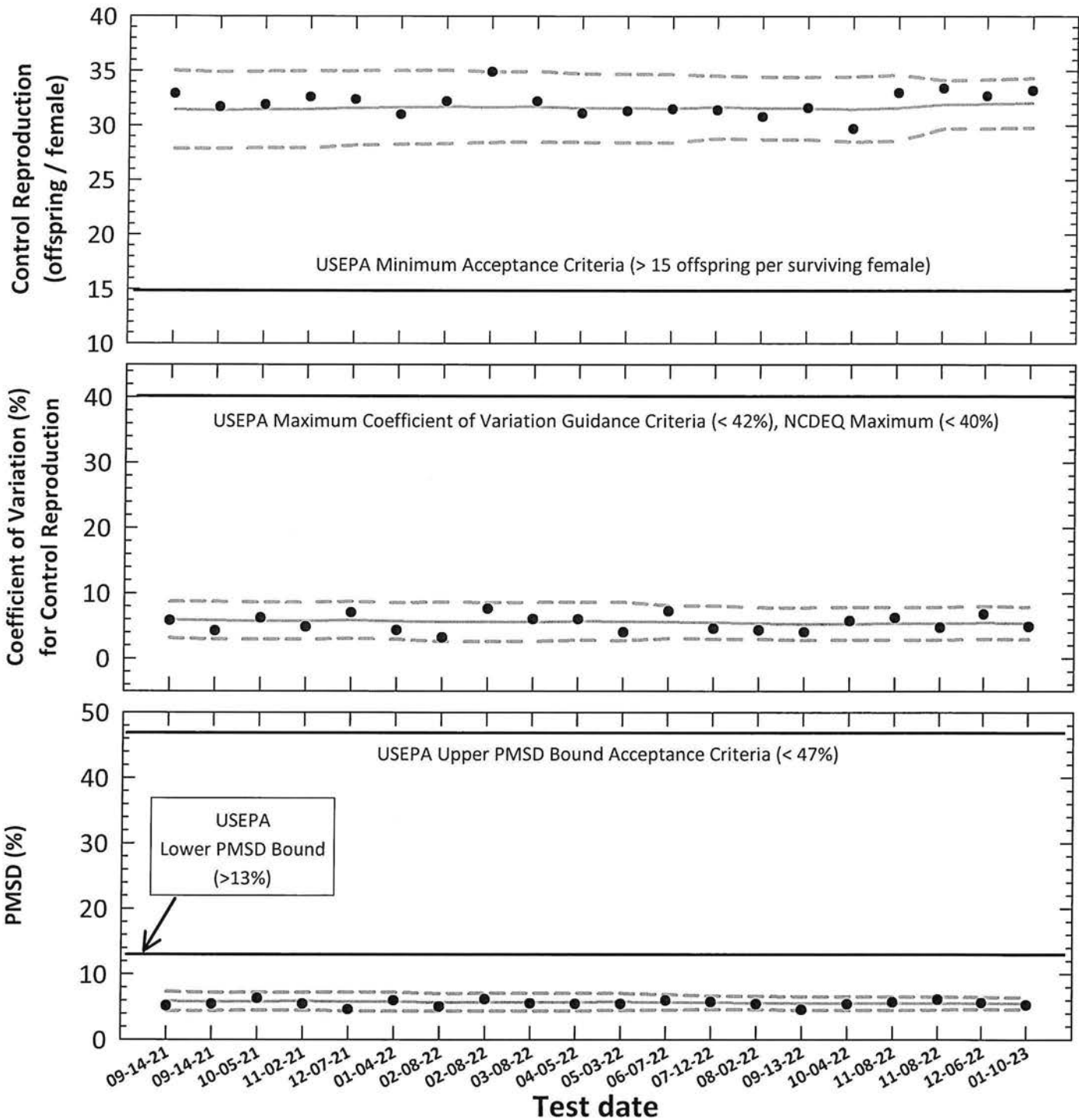
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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)

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

Test number	Test date	ToxCal Determination				Control Reproduction		Control Reproduction CV		Test PMSD					
		Control Survival (%)	Control Reproduction (offspring/female)		CT	95% Confidence Interval		CT	95% Confidence Interval						
			Mean	CV (%)		MSD	PMSD (%)		CT - 2S	CT + 2S	CT - 2S	CT + 2S			
1	09-14-21	100	32.9	5.8	1.698	5.2	31.4	27.9	35.0	5.9	8.7	5.9	8.7	4.4	7.3
2	09-14-21	100	31.7	4.2	1.729	5.5	31.4	27.9	34.9	5.8	8.7	5.8	8.7	4.4	7.2
3	10-05-21	100	31.9	6.2	2.014	6.3	31.5	27.9	35.0	5.7	8.6	5.8	8.6	4.5	7.2
4	11-02-21	100	32.6	4.8	1.782	5.5	31.5	27.9	35.0	5.7	8.6	5.9	8.6	4.5	7.2
5	12-07-21	100	32.4	7.0	1.499	4.6	31.6	28.2	35.0	5.9	8.7	5.8	8.7	4.4	7.3
6	01-04-22	100	31.0	4.3	1.854	6.0	31.6	28.3	35.0	5.7	8.5	5.8	8.5	4.4	7.2
7	02-08-22	100	32.2	3.2	1.623	5.0	31.7	28.3	35.1	5.6	8.6	5.7	8.6	4.4	7.0
8	02-08-22	100	34.9	7.6	2.146	6.1	31.7	28.5	34.9	5.6	8.5	5.7	8.5	4.4	7.1
9	03-08-22	100	32.2	6.0	1.773	5.5	31.7	28.5	34.9	5.6	8.6	5.7	8.6	4.4	7.1
10	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	8.6	5.7	8.6	4.4	7.0
11	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	8.6	5.7	8.6	4.4	7.1
12	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	8.1	5.7	8.1	4.5	6.9
13	07-12-22	100	31.4	4.6	1.804	5.7	31.7	28.8	34.5	5.5	8.0	5.6	8.0	4.6	6.7
14	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	7.8	5.6	7.8	4.6	6.6
15	09-13-22	100	31.6	4.0	1.437	4.5	31.6	28.7	34.4	5.3	7.8	5.5	7.8	4.5	6.6
16	10-04-22	100	29.7	5.7	1.610	5.4	31.5	28.5	34.5	5.3	7.8	5.5	7.8	4.5	6.6
17	11-08-22	100	33.0	6.2	1.880	5.7	31.6	28.6	34.6	5.3	7.8	5.5	7.8	4.5	6.6
18	11-08-22	100	33.4	4.7	2.044	6.1	31.9	29.7	34.1	5.3	7.8	5.5	7.8	4.5	6.5
19	12-06-22	100	32.7	6.8	1.830	5.6	32.0	29.7	34.2	5.5	8.0	5.5	8.0	4.6	6.5
20	01-10-23	100	33.2	4.9	1.756	5.3	32.1	29.8	34.3	5.4	7.9	5.5	7.9	4.6	6.4

Note:

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

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

CV = Coefficient of variation for control reproduction.

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test. Lower PMSD bound determined by USEPA (10th percentile) > 13%.

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

Upper PMSD bound 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*

CdNaCICR #: 280

Dilution preparation information:						Comments:
NaCl Stock INSS number:	INSS <u>2149</u>					
Stock preparation:	100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.					
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Test randomization and location:

Organism age:	< 24-hours old	Randomizing template color:	<u>GREEN</u>
Date and times organisms were born between:	<u>01-10-23 0530 TO 0750</u>	Incubator number and shelf location:	<u>2B1</u>
Culture board:	<u>01-03-23 A</u>		
Replicate number:	1 2 3 4 5 6 7 8 9 10		
Culture board cup number:	<u>3 4 5 10 14 15 18 20 23 24</u>		
Transfer vessel information:	pH (S.U.): <u>8.03</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	01-10-23	<u>0800</u>	<u>12-27-22</u>	<u>01-03-23</u>	<u>01-04-23B</u>	<u>JL</u>
1	01-11-23	<u>0800</u>			↓	<u>JL</u>
2	01-12-23	<u>0800</u>			<u>01-04-23C</u>	<u>JL</u>
3	01-13-23	<u>0800</u>			↓	<u>JL</u>
4	01-14-23	<u>0900</u>			<u>01-04-23E</u>	<u>JL</u>
5	01-15-23	<u>0853</u>			↓	<u>JL</u>
6	01-16-23	<u>0805</u>			↓	<u>JL</u>
7	01-17-23	<u>0736</u>			↓	<u>JL</u>

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

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	<u>71400</u>
% Adults having 3 rd Broods:	<u>1007.</u>	≥ 80%	NOEC (mg/L NaCl)	<u>1000</u>
% Mortality:	<u>07.</u>	≤ 20%	LOEC (mg/L NaCl)	<u>1200</u>
Mean Offspring/Female:	<u>33.2</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.5</u>
% CV:	<u>4.97.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1076.7</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 280

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	4	6	4	4	4	5	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	13	12	12	10	12	13	12	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	19	15	15	19	14	18	18	14	17	16
Total young produced		34	32	34	35	30	32	35	32	34	34
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	33.2

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	6	4	4	5	6	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	13	11	13	13	13	12	12	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	17	19	15	19	13	17	18	15	19
Total young produced		32	34	38	30	36	31	36	35	32	35
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	33.9
% Reduction from Control:	-2.17.

Species: Ceriodaphnia dubia
800 mg NaCl/L

CdNaClCR #: 280

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	5	5	4	4	5	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	11	12	12	10	13	13	13	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	18	15	19	15	15	15	16	13	16
Total young produced		32	31	31	36	31	29	33	33	30	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	31.7
% Reduction from Control:	45.7.

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	5	4	5	5	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	10	10	12	12	11	12	11	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	16	16	14	14	14	15	12	15	15	12
Total young produced		30	29	28	31	30	31	29	30	31	29
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	29.8
% Reduction from Control:	10.27.

Species: *Ceriodaphnia dubia*
1200 mg NaCl/L

CdNaCICR #: 280

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	4	4	5	4	3	3	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	7	9	5	8	5	9	7	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	5	9	4	8	3	10	4	4	8	9
Total young produced		18	20	17	18	15	18	16	16	20	19
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:	17.7
% Reduction from Control:	46.77.

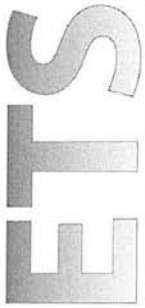
1400 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	1.4
% Reduction from Control:	94.37.



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	4	6	4	4	4	5	5	5	5	47
5	10	13	13	12	12	10	12	13	12	13	120
6	0	0	0	0	0	0	0	0	0	0	0
7	19	15	15	19	14	18	18	14	17	16	165
Total	34	32	34	35	30	32	35	32	34	34	332

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	6	4	4	5	6	5	5	5	50
5	10	12	13	11	13	13	13	12	12	11	120
6	0	0	0	0	0	0	0	0	0	0	0
7	17	17	19	15	19	13	17	18	15	19	169
Total	32	34	38	30	36	31	36	35	32	35	339

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	5	5	4	4	5	4	4	4	43
5	12	10	11	12	12	10	13	13	13	11	117
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	15	19	15	15	15	16	13	16	157
Total	32	31	31	36	31	29	33	33	30	31	317

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	5	4	5	4	5	4	4	42
5	10	10	10	12	12	11	12	11	12	13	113
6	0	0	0	0	0	0	0	0	0	0	0
7	16	16	14	14	14	15	12	15	15	12	143
Total	30	29	28	31	30	31	29	30	31	29	298

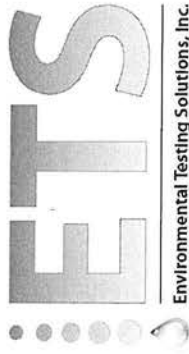
1200 mg NaCl/L

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	5	4	3	3	5	5	4	40
5	10	7	9	5	8	5	9	7	7	6	73
6	0	0	0	0	0	0	0	0	0	0	0
7	5	9	4	8	3	10	4	4	8	9	64
Total	18	20	17	18	15	18	16	16	20	19	177

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	3	1	2	1	1	3	1	1	16
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	2	0	0	0	0	1	3
Total	2	1	3	3	2	1	1	3	1	2	19

Retired and
no longer
in service



***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 #280
 Test dates: January 10-17, 2023

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	34	32	34	35	30	32	35	32	34	34	100	33.2	4.9	Not applicable
600	32	34	38	30	36	31	36	35	32	35	100	33.9	7.5	-2.1
800	32	31	31	36	31	29	33	33	30	31	100	31.7	6.1	4.5
1000	30	29	28	31	30	31	29	30	31	29	100	29.8	3.5	10.2
1200	18	20	17	18	15	18	16	16	20	19	100	17.7	9.6	46.7
1400	2	1	3	3	2	1	1	3	1	2	100	1.9	46.1	94.3

Dunnnett's MSD value: 1.756
 PMSD: 5.3

MSD = Minimum Significant Difference
 PMSD = Percent Minimum Significant Difference

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

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

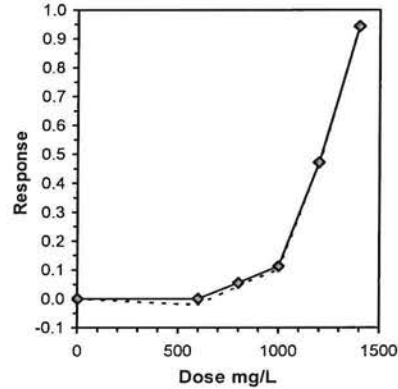
Start Date: 1/10/2023 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant
 End Date: 1/17/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia
 Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	34.000	32.000	34.000	35.000	30.000	32.000	35.000	32.000	34.000	34.000
600	32.000	34.000	38.000	30.000	36.000	31.000	36.000	35.000	32.000	35.000
800	32.000	31.000	31.000	36.000	31.000	29.000	33.000	33.000	30.000	31.000
1000	30.000	29.000	28.000	31.000	30.000	31.000	29.000	30.000	31.000	29.000
1200	18.000	20.000	17.000	18.000	15.000	18.000	16.000	16.000	20.000	19.000
1400	2.000	1.000	3.000	3.000	2.000	1.000	1.000	3.000	1.000	2.000

Conc-mg/L	Transform: Untransformed							N	1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	t-Stat		Critical	MSD	Mean	N-Mean	
D-Control	33.200	1.0000	33.200	30.000	35.000	4.877	10				33.550	1.0000	
600	33.900	1.0211	33.900	30.000	38.000	7.546	10	-0.912	2.287	1.756	33.550	1.0000	
800	31.700	0.9548	31.700	29.000	36.000	6.140	10	1.953	2.287	1.756	31.700	0.9449	
*1000	29.800	0.8976	29.800	28.000	31.000	3.466	10	4.428	2.287	1.756	29.800	0.8882	
*1200	17.700	0.5331	17.700	15.000	20.000	9.621	10	20.186	2.287	1.756	17.700	0.5276	
*1400	1.900	0.0572	1.900	1.000	3.000	46.084	10	40.762	2.287	1.756	1.900	0.0566	

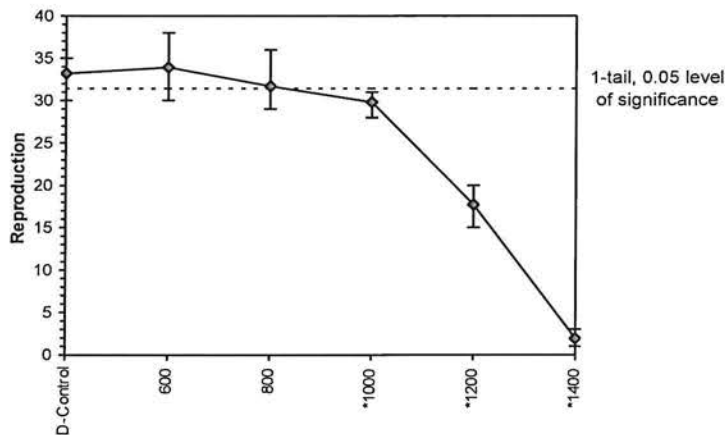
Auxiliary Tests				Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)				0.77147	1.035	0.08734	0.32093
Bartlett's Test indicates equal variances (p = 0.03)				12.4992	15.0863		
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU
Dunnett's Test				800	1000	894.427	
Treatments vs D-Control				MSDu	MSDp	MSB	MSE
				1.75587	0.05289	1601.48	2.94815
				F-Prob	df		
				7.0E-45	5, 54		

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL	Skew	
IC05	781.351	53.7216	691.32	877.72	0.2379
IC10	958.421	51.7561	797.119	1007.49	-1.0619
IC15	1021.2	8.04902	1006.35	1034.92	-0.3317
IC20	1048.93	7.28529	1034.41	1062.12	0.0599
IC25	1076.65	7.03373	1063.39	1089.97	0.1069
IC40	1159.83	7.84654	1145.2	1173.93	0.2148
IC50	1211.71	6.46557	1198.42	1222.44	-0.1695



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

Dose-Response Plot



Entered and Reviewed by Jim Sumner

Species: Ceriodaphnia dubia

CdNaClCR #: 280

Daily Chemistry:

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

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		EC	EC μ	EC μ	μ	μ	μ
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.01	7.81	7.83	7.06	7.07	8.00
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.9	7.7	7.6
	Conductivity (μ mhos/cm)	300		313		298	
	Alkalinity (mg CaCO ₃ /L)	63				63	
	Hardness (mg CaCO ₃ /L)	86				88	
	Temperature (°C)	24.9	25.1	24.8	24.9	24.9	25.1
600 mg NaCl/L	pH (S.U.)	8.00	7.82	7.80	7.04	7.96	7.95
	Dissolved oxygen (mg/L)	7.7	7.0	7.6	7.9	7.7	7.6
	Conductivity (μ mhos/cm)	1490		1490		1400	
	Temperature (°C)	25.0	24.9	24.9	25.1	25.0	25.2
800 mg NaCl/L	pH (S.U.)	8.05	7.83	7.88	7.03	7.97	7.95
	Dissolved oxygen (mg/L)	7.7	7.6	7.6	7.9	7.0	7.6
	Conductivity (μ mhos/cm)	1850		1800		1770	
	Temperature (°C)	25.0	24.9	24.9	25.1	25.0	25.0
1000 mg NaCl/L	pH (S.U.)	8.05	7.84	7.89	7.05	7.97	7.96
	Dissolved oxygen (mg/L)	7.8	7.0	7.7	7.9	7.0	7.6
	Conductivity (μ mhos/cm)	2240		2200		2130	
	Temperature (°C)	25.0	24.9	24.9	24.8	25.0	25.0
1200 mg NaCl/L	pH (S.U.)	8.05	7.84	7.89	7.04	7.98	7.95
	Dissolved oxygen (mg/L)	7.8	7.6	7.7	7.9	7.7	7.5
	Conductivity (μ mhos/cm)	2640		2620		2510	
	Temperature (°C)	24.9	25.2	25.0	24.8	25.0	25.2
1400 mg NaCl/L	pH (S.U.)	8.05	7.84	7.88	7.04	7.97	7.95
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	7.8	7.7	7.5
	Conductivity (μ mhos/cm)	3050		2970		2840	
	Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.2
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

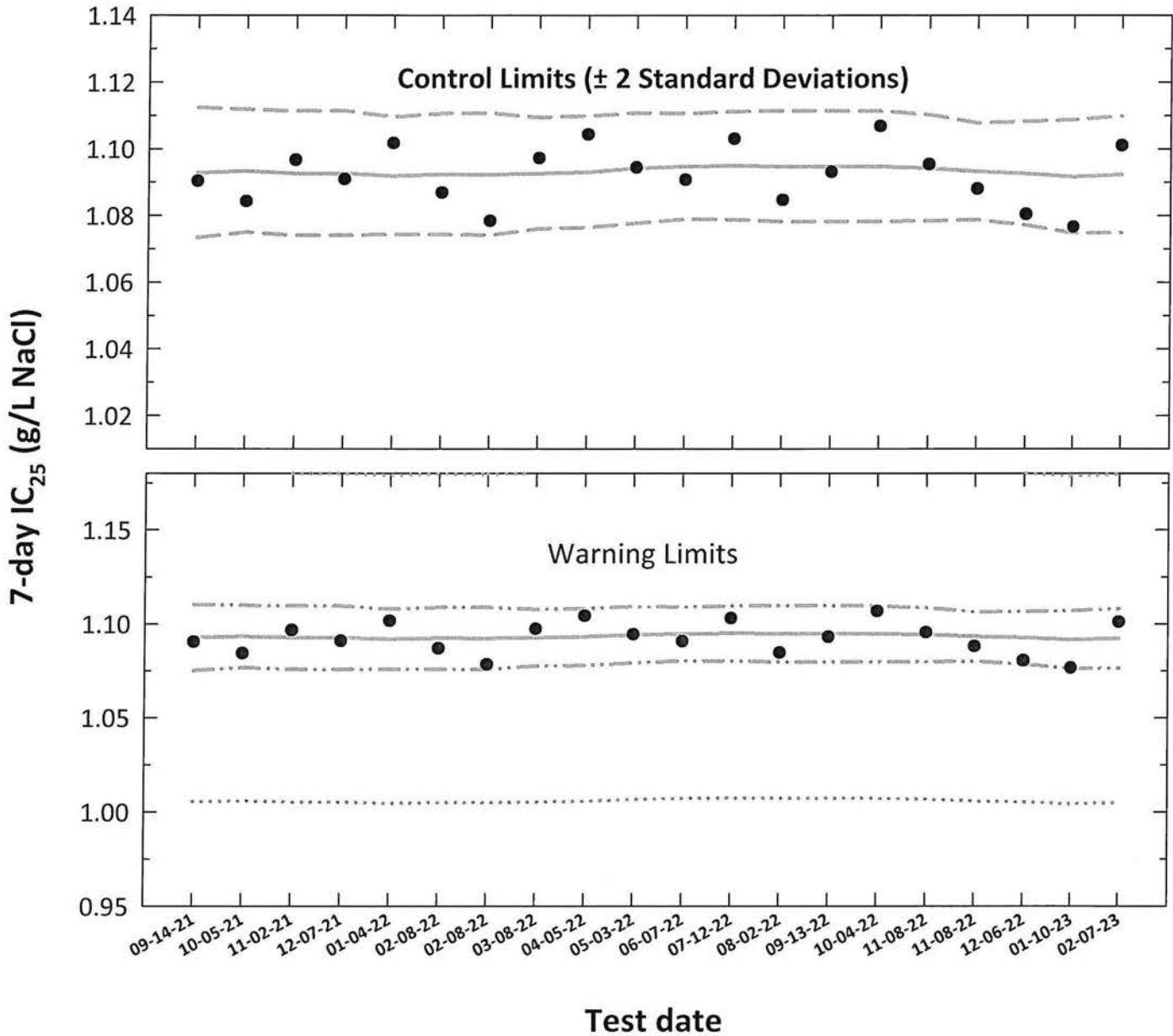
CdNaClCR #: 280

Analyst		Day							
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
Concentration	Parameter	W	BSC	BSC	W	W	EL	EL	W
CONTROL, MHSW	pH (S.U.)	8.07	8.04	7.97	7.94	8.00	7.88	7.92	7.88
	Dissolved oxygen (mg/L)	7.6	7.9	7.8	7.9	7.8	7.9	7.8	7.6
	Conductivity (µmhos/cm)	304		289		300		300	
	Alkalinity (mg CaCO ₃ /L)			62					
	Hardness (mg CaCO ₃ /L)			86					
	Temperature (°C)	24.8	25.2	24.9	25.1	24.6	24.8	24.9	25.3
600 mg NaCl/L	pH (S.U.)	8.07	8.05	8.04	7.92	8.00	7.94	7.95	7.85
	Dissolved oxygen (mg/L)	7.6	7.9	7.9	7.9	7.6	7.9	7.7	7.6
	Conductivity (µmhos/cm)	1420		1350		1300		1400	
	Temperature (°C)	24.8	25.2	24.9	24.8	24.7	24.9	25.0	25.1
800 mg NaCl/L	pH (S.U.)	8.00	8.05	8.08	7.92	8.00	7.92	7.97	7.87
	Dissolved oxygen (mg/L)	7.6	7.9	7.9	7.9	7.6	7.9	7.8	7.6
	Conductivity (µmhos/cm)	1790		1670		1730		1810	
	Temperature (°C)	24.8	25.0	25.0	24.8	24.7	24.9	25.0	25.2
1000 mg NaCl/L	pH (S.U.)	8.07	8.05	8.08	7.92	8.01	7.92	7.97	7.87
	Dissolved oxygen (mg/L)	7.6	7.9	8.0	7.9	7.6	7.9	7.7	7.6
	Conductivity (µmhos/cm)	2170		2030		2100		2170	
	Temperature (°C)	24.8	25.2	25.0	25.0	24.7	24.9	24.9	25.2
1200 mg NaCl/L	pH (S.U.)	8.00	8.04	8.09	7.92	8.01	7.92	7.99	7.87
	Dissolved oxygen (mg/L)	7.5	7.9	8.0	8.0	7.7	7.9	7.8	7.6
	Conductivity (µmhos/cm)	2520		2420		2460		2570	
	Temperature (°C)	24.9	24.9	25.0	25.2	24.8	25.1	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	8.00	8.05	8.09	7.96	8.02	7.93	7.99	7.87
	Dissolved oxygen (mg/L)	7.5	7.9	8.0	8.0	7.8	8.0	7.8	7.7
	Conductivity (µmhos/cm)	2890		2780		2820		2970	
	Temperature (°C)	24.9	24.9	25.1	25.2	24.7	24.9	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



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

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV			
							CT - 2S	CT + 2S	Warning Limits	Warning Limits		
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	07-12-22	1.1030	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
13	08-02-22	1.0847	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
14	09-13-22	1.0931	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
15	10-04-22	1.1068	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823
16	11-08-22	1.0954	0.0396	0.0391	0.0032	1.0943	1.0785	1.1103	1.0798	1.1087	1.0067	1.1818
17	11-08-22	1.0881	0.0367	0.0387	0.0029	1.0932	1.0788	1.1078	1.0800	1.1064	1.0057	1.1807
18	12-06-22	1.0805	0.0336	0.0385	0.0031	1.0926	1.0772	1.1083	1.0785	1.1068	1.0052	1.1800
19	01-10-23	1.0767	0.0321	0.0381	0.0034	1.0916	1.0748	1.1088	1.0762	1.1071	1.0043	1.1790
20	02-07-23	1.1011	0.0418	0.0383	0.0035	1.0922	1.0749	1.1098	1.0764	1.1081	1.0049	1.1796

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

CT = Central tendency of the IC₂₅ values.

S = Standard deviation of the IC₂₅ values.

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

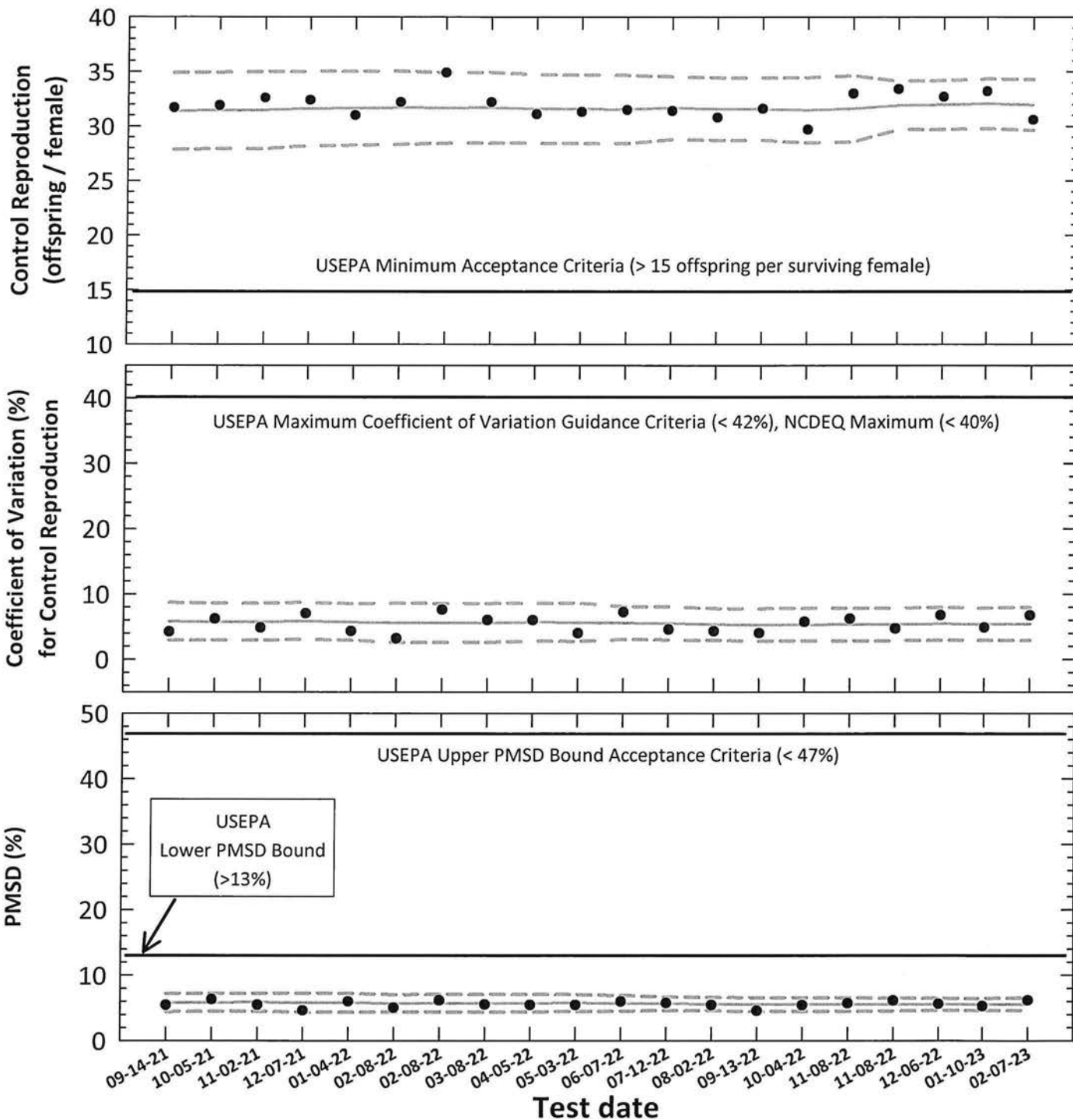
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Ceriodaphnia dubia

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



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

Entered and Reviewed by
Jim Sumner

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Ceriodaphnia dubia

Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Reproduction			Control Reproduction CV			Test PMSD		
		Control Survival (%)	Control Reproduction (offspring/female)		Test		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean	CV (%)	MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	09-14-21	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.7	8.6	5.7	4.4	7.0
10	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.5	3.0	8.1	5.7	4.5	6.9
11	06-07-22	100	31.5	7.2	1.876	6.0	31.5	28.4	34.7	5.5	3.0	8.1	5.7	4.5	6.9
12	07-12-22	100	31.4	4.6	1.804	5.7	31.7	28.8	34.5	5.5	2.9	8.0	5.6	4.6	6.7
13	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	2.9	7.8	5.6	4.6	6.6
14	09-13-22	100	31.6	4.0	1.437	4.5	31.6	28.7	34.4	5.3	2.8	7.8	5.5	4.5	6.6
15	10-04-22	100	29.7	5.7	1.610	5.4	31.5	28.5	34.5	5.3	2.8	7.8	5.5	4.5	6.6
16	11-08-22	100	33.0	6.2	1.880	5.7	31.6	28.6	34.6	5.3	2.8	7.8	5.5	4.5	6.6
17	11-08-22	100	33.4	4.7	2.044	6.1	31.9	29.7	34.1	5.3	2.8	7.8	5.5	4.5	6.5
18	12-06-22	100	32.7	6.8	1.830	5.6	32.0	29.7	34.2	5.5	2.9	8.0	5.5	4.6	6.5
19	01-10-23	100	33.2	4.9	1.756	5.3	32.1	29.8	34.3	5.4	2.9	7.9	5.5	4.6	6.4
20	02-07-23	100	30.6	6.8	1.891	6.2	32.0	29.6	34.3	5.4	2.9	8.0	5.6	4.6	6.5

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

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

CV = Coefficient of variation for control reproduction.

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

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.

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

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

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

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

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

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

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

CdNaClCR #: 281

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>267</u>				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Test randomization and location:

Organism age:	< 24-hours old										Randomizing template color:	<u>Blue</u>
Date and times organisms were born between:	<u>02-07-23 0520 TO 0815</u>										Incubator number and shelf location:	<u>2B1</u>
Culture board:	<u>01-31-23 A</u>											
Replicate number:	1	2	3	4	5	6	7	8	9	10		
Culture board cup number:	<u>5</u>	<u>8</u>	<u>11</u>	<u>12</u>	<u>17</u>	<u>25</u>	<u>28</u>	<u>29</u>	<u>30</u>	<u>38</u>		
Transfer vessel information:	pH (S.U.): <u>7.66</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	02-07-23	<u>0827</u>	<u>02-07-23</u>	<u>02-07-23</u>	<u>02-01-23B</u>	<u>JH</u>
1	02-08-23	<u>0740</u>	↓	↓	↓	<u>JH</u>
2	02-09-23	<u>0741</u>	↓	↓	<u>02-01-23D</u>	<u>JH</u>
3	02-10-23	<u>0740</u>	↓	↓	↓	<u>JH</u>
4	02-11-23	<u>0840</u>	↓	↓	<u>02-09-23A</u>	<u>JH</u>
5	02-12-23	<u>0740</u>	↓	↓	↓	<u>JH</u>
6	02-13-23	<u>0740</u>	↓	↓	↓	<u>JH</u>
7	02-14-23	<u>0728</u>	↓	↓	↓	<u>JH</u>

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

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	<u>71400</u>
% Adults having 3 rd Broods:	<u>1007.</u>	≥ 80%	NOEC (mg/L NaCl)	<u>1000</u>
% Mortality:	<u>07.</u>	≤ 20%	LOEC (mg/L NaCl)	<u>1200</u>
Mean Offspring/Female:	<u>30.6</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.5</u>
% CV:	<u>6.87.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1101.1</u>

Species: Ceriodaphnia dubia

CdNaClCR #: 281

CONTROL

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	6	4	5	4	5	5	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	11	11	11	9	12	10	13	10	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	17	16	14	15	12	18	15	15	13
Total young produced		29	34	31	30	28	29	33	33	30	29
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	30.6

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	6	5	5	4	5	5	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	13	12	13	10	10	12	11	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	14	15	18	17	15	15	13	16	15
Total young produced		30	33	32	36	31	30	32	28	33	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:	-2.97.

Species: *Ceriodaphnia dubia*
800 mg NaCl/L

CdNaClCR #: 281

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	5	5	6	4	4	5	5	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	10	12	11	11	12	10	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	16	13	15	16	12	15	14	14	17
Total young produced		32	29	28	32	33	27	31	29	29	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	30.3
% Reduction from Control:	1.07.

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	5	4	5	5	5	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	11	11	10	10	12	10	10	10	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	13	16	14	16	12	17	13	14	14
Total young produced		26	28	32	28	31	29	32	28	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:	07.
Mean Offspring/Female:	29.2
% Reduction from Control:	4.67.

Species: Ceriodaphnia dubia
1200 mg NaCl/L

CdNaCICR #: 281

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	17.5
% Reduction from Control:	42.87.

1400 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	2.2
% Reduction from Control:	92.87.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	4	5	4	5	5	5	4	4	47
5	10	11	11	9	12	10	13	10	12	10	109
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	16	14	15	12	18	15	15	13	150
Total	29	34	31	30	28	29	33	33	30	29	306

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	5	5	4	5	5	4	5	5	48
5	11	13	12	13	10	10	12	11	12	10	114
6	0	0	0	0	0	0	0	0	0	0	0
7	15	14	15	18	17	15	15	13	16	15	153
Total	30	33	32	36	31	30	32	28	33	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	3	5	5	6	4	4	5	5	6	48
5	11	10	10	12	11	11	12	10	10	10	107
6	0	0	0	0	0	0	0	0	0	0	0
7	16	16	13	15	16	12	15	14	14	17	148
Total	32	29	28	32	33	27	31	29	29	33	303

1000 mg NaCl/L

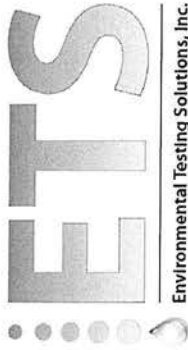
Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	4	5	4	5	5	5	5	4	46
5	9	11	11	10	10	12	10	10	10	11	104
6	0	0	0	0	0	0	0	0	0	0	0
7	13	13	16	14	16	12	17	13	14	14	142
Total	26	28	32	28	31	29	32	28	29	29	292

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	4	4	4	4	4	3	3	4	37
5	10	7	9	5	6	5	9	5	7	7	70
6	0	0	0	0	0	0	0	0	0	0	0
7	4	8	5	9	9	7	6	8	5	7	68
Total	17	19	18	18	19	16	19	16	15	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	3	2	2	1	3	3	1	1	3	21
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	1	0	0	0	0	0	0	1
Total	2	3	2	3	1	3	3	1	1	3	22



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 #281**
 Test dates: **February 07-14, 2023**

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	29	34	31	30	28	29	33	33	30	29	100	30.6	6.8	Not applicable
600	30	33	32	36	31	30	32	28	33	30	100	31.5	7.1	-2.9
800	32	29	28	32	33	27	31	29	29	33	100	30.3	7.1	1.0
1000	26	28	32	28	31	29	32	28	29	29	100	29.2	6.6	4.6
1200	17	19	18	18	19	16	19	16	15	18	100	17.5	8.2	42.8
1400	2	3	2	3	1	3	3	1	1	3	100	2.2	41.8	92.8

Dunnett's MSD value: 1.891
 PMSD: 6.2

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.



Statistical Analyses

Ceriodaphnia Survival and Reproduction Test-Reproduction

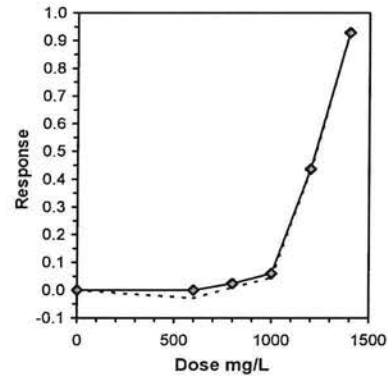
Start Date: 2/7/2023	Test ID: CdNaClCR	Sample ID: REF-Ref Toxicant
End Date: 2/14/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: NaCl-Sodium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: CD-Ceriodaphnia dubia
Comments:		

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	29,000	34,000	31,000	30,000	28,000	29,000	33,000	33,000	30,000	29,000
600	30,000	33,000	32,000	36,000	31,000	30,000	32,000	28,000	33,000	30,000
800	32,000	29,000	28,000	32,000	33,000	27,000	31,000	29,000	29,000	33,000
1000	26,000	28,000	32,000	28,000	31,000	29,000	32,000	28,000	29,000	29,000
1200	17,000	19,000	18,000	18,000	19,000	16,000	19,000	16,000	15,000	18,000
1400	2,000	3,000	2,000	3,000	1,000	3,000	3,000	1,000	1,000	3,000

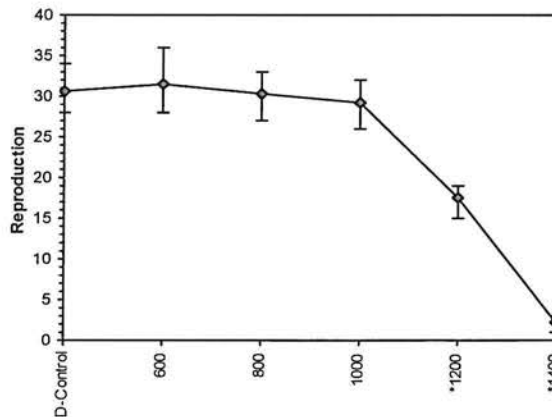
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
D-Control	30.600	1.0000	30.600	28.000	34.000	6.750	10			31.050	1.0000
600	31.500	1.0294	31.500	28.000	36.000	7.059	10	117.00	75.00	31.050	1.0000
800	30.300	0.9902	30.300	27.000	33.000	7.138	10	99.50	75.00	30.300	0.9758
1000	29.200	0.9542	29.200	26.000	32.000	6.617	10	84.50	75.00	29.200	0.9404
*1200	17.500	0.5719	17.500	15.000	19.000	8.193	10	55.00	75.00	17.500	0.5636
*1400	2.200	0.0719	2.200	1.000	3.000	41.770	10	55.00	75.00	2.200	0.0709

Auxiliary Tests				Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution ($p \leq 0.01$)				1.0554	1.035	0.23324	-0.3882
Bartlett's Test indicates equal variances ($p = 0.16$)				7.91807	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							

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL	Skew	
IC05	945.909	80.621	750.06	1013.34	-0.9882
IC10	1021.45	16.8179	976.121	1038.06	-3.5198
IC15	1047.99	10.1808	1022.76	1063.73	-0.5947
IC20	1074.53	9.25102	1053.19	1089.24	-0.4942
IC25	1101.07	8.55429	1083.7	1115.18	-0.3431
IC40	1180.68	8.35842	1165.46	1197.71	0.1075
IC50	1225.82	5.87474	1214.3	1236.12	-0.1519



Dose-Response Plot



Entered and
Reviewed by
Jim Sumner
JS



Statistical Analyses

Used for PMSD calculation only.

Ceriodaphnia Survival and Reproduction Test-Reproduction

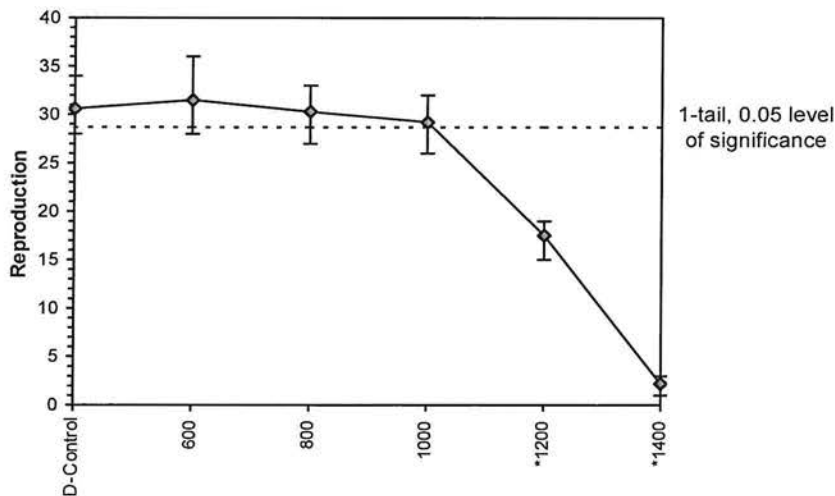
Start Date: 2/7/2023 Test ID: CdNaCICR Sample ID: REF-Ref Toxicant
 End Date: 2/14/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: NACL-Sodium chloride
 Sample Date: Protocol: FWCHR-EPA-821-R-02-013 Test Species: CD-Ceriodaphnia dubia
 Comments:

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	29.000	34.000	31.000	30.000	28.000	29.000	33.000	33.000	30.000	29.000
600	30.000	33.000	32.000	36.000	31.000	30.000	32.000	28.000	33.000	30.000
800	32.000	29.000	28.000	32.000	33.000	27.000	31.000	29.000	29.000	33.000
1000	26.000	28.000	32.000	28.000	31.000	29.000	32.000	28.000	29.000	29.000
1200	17.000	19.000	18.000	18.000	19.000	16.000	19.000	16.000	15.000	18.000
1400	2.000	3.000	2.000	3.000	1.000	3.000	3.000	1.000	1.000	3.000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD
			Mean	Min	Max	CV%					
D-Control	30.600	1.0000	30.600	28.000	34.000	6.750	10				
600	31.500	1.0294	31.500	28.000	36.000	7.059	10	-1.088	2.287	1.891	
800	30.300	0.9902	30.300	27.000	33.000	7.138	10	0.363	2.287	1.891	
1000	29.200	0.9542	29.200	26.000	32.000	6.617	10	1.693	2.287	1.891	
*1200	17.500	0.5719	17.500	15.000	19.000	8.193	10	15.839	2.287	1.891	
*1400	2.200	0.0719	2.200	1.000	3.000	41.770	10	34.337	2.287	1.891	

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates non-normal distribution ($p \leq 0.01$)	1.0554	1.035	0.23324	-0.3882						
Bartlett's Test indicates equal variances ($p = 0.16$)	7.91807	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.89127	0.06181	1365.63	3.42037	2.5E-41	5, 54
Treatments vs D-Control										

Dose-Response Plot



Entered and Reviewed by
 Jim Sumner
JS

Species: Ceriodaphnia dubia

CdNaClCR #: 281

Daily Chemistry:

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

Concentration		Parameter	Day					
			(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
			0		1		2	
Analyst		u	u	u	u	u	u	
CONTROL, MHSW	pH (S.U.)	7.38	7.44	7.41	7.57	7.38	8.01	
	Dissolved oxygen (mg/L)	7.6	7.9	7.8	7.9	8.0	7.7	
	Conductivity (µmhos/cm)	293		320		317		
	Alkalinity (mg CaCO ₃ /L)	60				61		
	Hardness (mg CaCO ₃ /L)	86				94		
	Temperature (°C)	24.9	25.1	24.8	24.9	24.8	25.2	
600 mg NaCl/L	pH (S.U.)	7.72	7.63	7.68	7.56	7.61	7.97	
	Dissolved oxygen (mg/L)	7.6	7.9	7.8	7.8	7.9	7.6	
	Conductivity (µmhos/cm)	1410		1460		1400		
	Temperature (°C)	25.0	24.9	24.8	25.1	24.9	24.8	
800 mg NaCl/L	pH (S.U.)	7.72	7.63	7.68	7.63	7.66	7.88	
	Dissolved oxygen (mg/L)	7.6	7.9	7.8	7.7	7.9	7.6	
	Conductivity (µmhos/cm)	1820		1800		1790		
	Temperature (°C)	24.9	24.9	24.8	25.1	24.8	24.8	
1000 mg NaCl/L	pH (S.U.)	7.75	7.67	7.64	7.60	7.64	7.89	
	Dissolved oxygen (mg/L)	7.6	7.9	7.9	7.7	7.9	7.7	
	Conductivity (µmhos/cm)	2150		2220		2160		
	Temperature (°C)	24.9	25.2	24.8	24.9	24.8	25.1	
1200 mg NaCl/L	pH (S.U.)	7.75	7.56	7.69	7.59	7.65	7.90	
	Dissolved oxygen (mg/L)	7.6	7.9	7.9	7.8	7.9	7.7	
	Conductivity (µmhos/cm)	2550		2590		2530		
	Temperature (°C)	25.0	25.0	24.9	24.9	25.0	25.1	
1400 mg NaCl/L	pH (S.U.)	7.70	7.69	7.72	7.66	7.65	7.90	
	Dissolved oxygen (mg/L)	7.7	7.9	7.9	7.8	7.8	7.8	
	Conductivity (µmhos/cm)	2960		2940		2890		
	Temperature (°C)	25.0	25.0	24.8	24.9	25.0	24.9	
		Initial	Final	Initial	Final	Initial	Final	

Species: Ceriodaphnia dubia

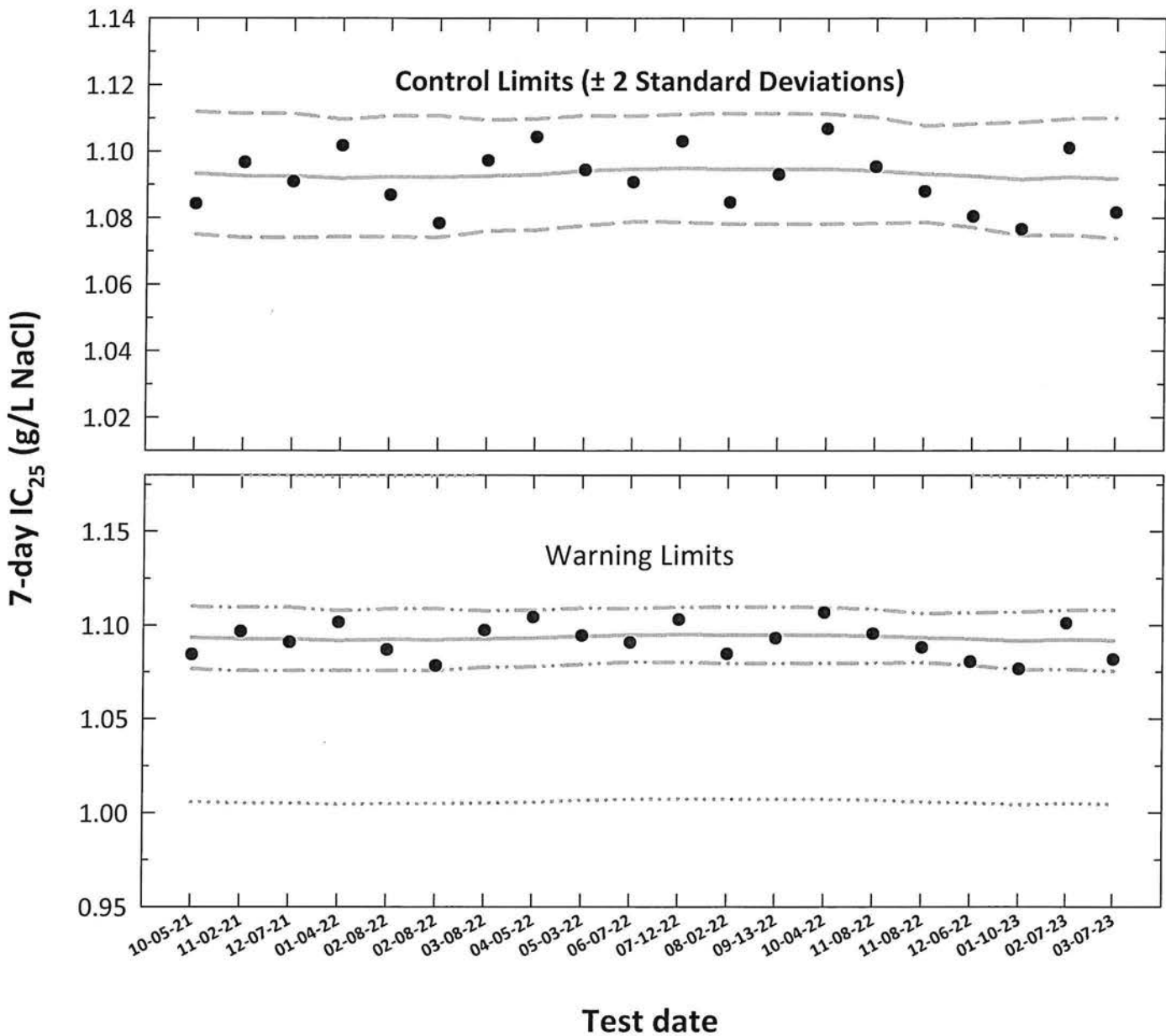
CdNaClCR #: 281

Concentration		Parameter		Day							
				(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
				3		4		5		6	
Analyst		U	BSC	BSC	U	U	U	U	U		
CONTROL, MHSW	pH (S.U.)	8.08	8.12	8.10	8.08	8.04	8.06	8.01	8.05		
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.7	7.6	7.8	7.7	7.9		
	Conductivity (µmhos/cm)	311		288		319		310			
	Alkalinity (mg CaCO ₃ /L)			61							
	Hardness (mg CaCO ₃ /L)			82							
	Temperature (°C)	24.8	25.2	24.8	25.1	24.9	25.0	24.9	25.2		
600 mg NaCl/L	pH (S.U.)	7.96	8.08	8.10	7.93	8.06	7.96	8.02	7.97		
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.7	7.6	7.6	7.6	7.6		
	Conductivity (µmhos/cm)	1390		1390		1490		1410			
	Temperature (°C)	24.9	24.8	24.8	24.9	24.8	24.9	24.9	24.9		
800 mg NaCl/L	pH (S.U.)	7.94	8.07	8.11	7.93	8.05	7.96	8.02	7.96		
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.6	7.5	7.6	7.6	7.7		
	Conductivity (µmhos/cm)	1780		1680		1710		1730			
	Temperature (°C)	24.9	24.8	24.9	24.9	24.8	24.9	25.0	24.9		
1000 mg NaCl/L	pH (S.U.)	7.93	8.06	8.11	7.93	8.05	7.96	8.03	7.95		
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.6	7.5	7.6	7.6	7.7		
	Conductivity (µmhos/cm)	2150		2060		2160		2070			
	Temperature (°C)	24.9	25.2	25.0	25.0	24.8	25.2	25.0	25.2		
1200 mg NaCl/L	pH (S.U.)	7.93	8.05	8.10	7.95	8.06	7.95	8.03	7.94		
	Dissolved oxygen (mg/L)	7.8	7.9	8.0	7.6	7.6	7.6	7.7	7.7		
	Conductivity (µmhos/cm)	2540		2500 2040 02-11-15		2610		2500			
	Temperature (°C)	25.0	25.2	25.0	25.0	25.9	24.9	24.9	25.1		
1400 mg NaCl/L	pH (S.U.)	7.96	8.04	8.08	7.99	8.06	7.95	8.03	7.94		
	Dissolved oxygen (mg/L)	7.8	8.0	8.0	7.6	7.6	7.6	7.7	7.8		
	Conductivity (µmhos/cm)	2950		2930		2930		2810			
	Temperature (°C)	25.0	24.9	25.0	25.0	24.9	25.0	25.0	24.9		
		Initial	Final	Initial	Final	Initial	Final	Initial	Final		

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture



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

Ceriodaphnia dubia

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L NaCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	10th Percentile CV Warning Limits CT - S _{A,10} CT + S _{A,10}			
1	10-05-21	1.0843	0.0376	0.0387	0.0037	1.0933	1.0750	1.1119	1.0766	1.1100	1.0058	1.1808
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	07-12-22	1.1030	0.0426	0.0394	0.0032	1.0949	1.0788	1.1113	1.0802	1.1096	1.0073	1.1825
12	08-02-22	1.0847	0.0353	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0072	1.1823
13	09-13-22	1.0931	0.0387	0.0393	0.0033	1.0947	1.0782	1.1115	1.0797	1.1098	1.0071	1.1823
14	10-04-22	1.1068	0.0441	0.0393	0.0033	1.0947	1.0783	1.1114	1.0797	1.1097	1.0071	1.1823
15	11-08-22	1.0954	0.0396	0.0391	0.0032	1.0943	1.0785	1.1103	1.0798	1.1087	1.0067	1.1818
16	11-08-22	1.0881	0.0367	0.0387	0.0029	1.0932	1.0788	1.1078	1.0800	1.1064	1.0057	1.1807
17	12-06-22	1.0805	0.0336	0.0385	0.0031	1.0926	1.0772	1.1083	1.0785	1.1068	1.0052	1.1800
18	01-10-23	1.0767	0.0321	0.0381	0.0034	1.0916	1.0748	1.1088	1.0762	1.1071	1.0043	1.1790
19	02-07-23	1.1011	0.0418	0.0383	0.0035	1.0922	1.0749	1.1098	1.0764	1.1081	1.0049	1.1796
20	03-07-23	1.0816	0.0341	0.0381	0.0036	1.0918	1.0739	1.1100	1.0754	1.1082	1.0045	1.1791

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

S = Standard deviation of the IC₂₅ values.

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

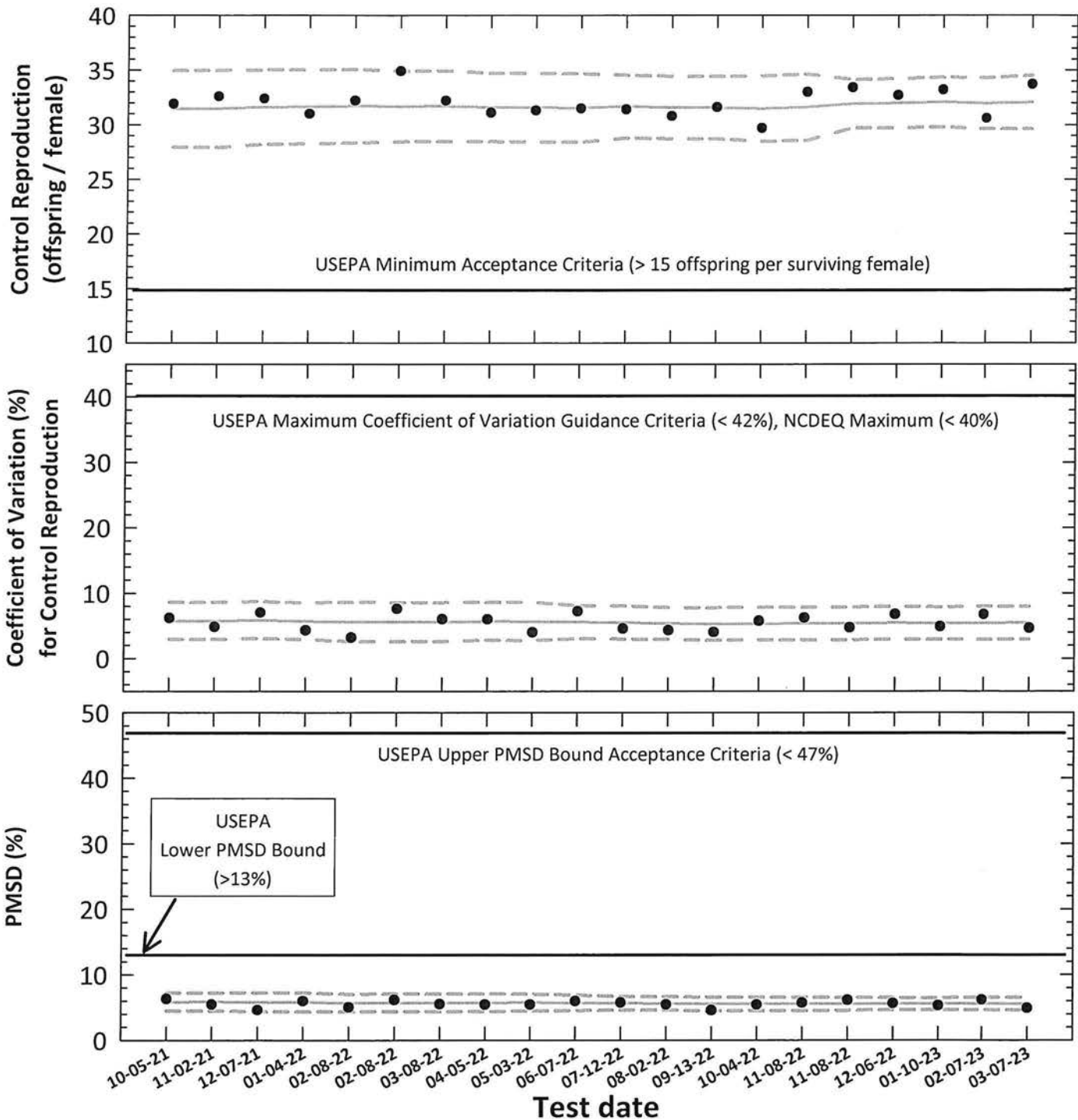
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,10} converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Ceriodaphnia dubia

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



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

Entered and
Reviewed by
Jim Sumner

Chronic Reference Toxicant Testing, Test Acceptability Criteria

Source: In-house Culture

Ceriodaphnia dubia

Test number	Test date	ToxCal Determination				Control Reproduction		Control Reproduction (offspring/female)		Control Reproduction CV		Test PMSD (%)						
		Control Survival (%)	Control Reproduction		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 (offspring/female)	CV (%)											MSD	Test		
1	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
2	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	2.9	8.6	5.9	4.5	7.2
3	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
4	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
5	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
6	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
7	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
8	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.7	8.6	5.7	2.7	8.6	5.7	4.4	7.0
9	05-03-22	100	31.3	4.0	1.707	5.5	31.5	28.4	34.7	5.5	3.0	8.1	5.7	3.0	8.1	5.7	4.5	6.9
10	06-07-22	100	31.5	7.2	1.876	6.0	31.7	28.8	34.5	5.5	2.9	8.0	5.6	2.9	8.0	5.6	4.6	6.7
11	07-12-22	100	31.4	4.6	1.804	5.7	31.6	28.7	34.4	5.3	2.9	7.8	5.6	2.9	7.8	5.5	4.5	6.6
12	08-02-22	100	30.8	4.3	1.676	5.4	31.6	28.7	34.4	5.3	2.8	7.8	5.5	2.8	7.8	5.5	4.5	6.6
13	09-13-22	100	31.6	4.0	1.437	4.5	31.6	28.7	34.4	5.3	2.8	7.8	5.5	2.8	7.8	5.5	4.5	6.6
14	10-04-22	100	29.7	5.7	1.610	5.4	31.5	28.5	34.5	5.3	2.8	7.8	5.5	2.8	7.8	5.5	4.5	6.6
15	11-08-22	100	33.0	6.2	1.880	5.7	31.6	28.6	34.6	5.3	2.8	7.8	5.5	2.8	7.8	5.5	4.5	6.6
16	11-08-22	100	33.4	4.7	2.044	6.1	31.9	29.7	34.1	5.3	2.8	7.8	5.5	2.8	7.8	5.5	4.5	6.5
17	12-06-22	100	32.7	6.8	1.830	5.6	32.0	29.7	34.2	5.5	2.9	8.0	5.5	2.9	8.0	5.5	4.6	6.5
18	01-10-23	100	33.2	4.9	1.756	5.3	32.1	29.8	34.3	5.4	2.9	7.9	5.5	2.9	7.9	5.5	4.6	6.4
19	02-07-23	100	30.6	6.8	1.891	6.2	32.0	29.6	34.3	5.4	2.9	8.0	5.6	2.9	8.0	5.6	4.6	6.5
20	03-07-23	100	33.7	4.7	1.648	4.9	32.1	29.6	34.5	5.4	2.9	8.0	5.5	2.9	8.0	5.5	4.5	6.5

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

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

CV = Coefficient of variation for control reproduction.

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%.

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 #: 282

Dilution preparation information:						Comments:
NaCl Stock INSS number:		INSS <u>2167</u>				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Test randomization and location:

Organism age:	< 24-hours old	Randomizing template color:	<u>GREEN</u>
Date and times organisms were born between:	<u>03-07-23 0530 TO 0805</u>	Incubator number and shelf location:	<u>2B1</u>
Culture board:	<u>02-28-23 A</u>		
Replicate number:	1 2 3 4 5 6 7 8 9 10		
Culture board cup number:	<u>2 3 7 12 13 15 19 24 27 28</u>		
Transfer vessel information:	pH (S.U.): <u>8.00</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	03-07-23	<u>0816</u>	<u>03-02-23</u>	<u>03-02-23</u>	<u>02-28-23 A</u>	<u>JL</u>
1	03-08-23	<u>0800</u>			↓	<u>JL</u>
2	03-09-23	<u>0800</u>			<u>02-28-23 D</u>	<u>JL</u>
3	03-10-23	<u>0751</u>			↓	<u>JL</u>
4	03-11-23	<u>0915</u>			<u>03-06-23 A</u>	<u>JL</u>
5	03-12-23	<u>0916</u>			↓	<u>JL</u>
6	03-13-23	<u>0751</u>	↓	↓	↓	<u>JL</u>
7	03-14-23	<u>0730</u>				<u>JL</u>

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

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% of Male Adults:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L NaCl)	<u>>1400</u>
% Adults having 3 rd Broods:	<u>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>33.7</u>	≥ 15.0 offspring/female	ChV (mg/L NaCl)	<u>1095.5</u>
% CV:	<u>4.67.</u>	< 40.0 %	IC ₂₅ (mg/L NaCl)	<u>1081.6</u>

Species: *Ceriodaphnia dubia*

CdNaClCR #: 282

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	4	5	5	6	5	5	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	13	14	12	10	12	12	12	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	17	15	16	14	19	17	16	17	19
Total young produced		36	32	33	35	32	31	34	32	33	36
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3 rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	33.7

600 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	34.5
% Reduction from Control:	-2.47.

Species: *Ceriodaphnia dubia*
800 mg NaCl/L

CdNaClCR #: 282

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	6	5	5	4	5	5	6	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	12	11	12	10	12	12	12	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	15	14	17	17	17	13	17	16	17
Total young produced		30	31	32	33	34	31	30	34	34	35
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	07.
Mean Offspring/Female:	32.4
% Reduction from Control:	3.9?

1000 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	5	5	3	5	5	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	10	10	12	11	12	12	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	14	14	18	13	16	15	15	17	13
Total young produced		29	30	29	33	28	32	32	32	34	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:	30.8
% Reduction from Control:	8.67.

Species: Ceriodaphnia dubia

CdNaClCR #: 282

1200 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	18.0
% Reduction from Control:	46.67

1400 mg NaCl/L

Survival and Reproduction Data

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	1.8
% Reduction from Control:	94.77

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	4	5	5	6	5	5	4	4	4	47
5	13	11	13	14	12	10	12	12	13	13	122
6	0	0	0	0	0	0	0	0	0	0	0
7	18	17	15	16	14	19	17	16	17	19	168
Total	36	32	33	35	32	34	34	32	33	36	337

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	5	5	3	5	5	5	5	4	45
5	10	12	10	10	12	11	12	12	12	12	113
6	0	0	0	0	0	0	0	0	0	0	0
7	15	14	14	18	13	16	15	15	17	13	150
Total	29	30	29	33	28	32	32	32	34	29	308

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	6	5	5	4	5	5	5	6	50
5	13	12	14	14	11	12	10	12	12	12	122
6	0	0	0	0	0	0	0	0	0	0	0
7	18	18	14	17	18	15	19	19	16	19	173
Total	36	34	34	36	34	31	34	36	33	37	345

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	3	5	4	4	4	4	6	4	41
5	8	5	8	9	6	6	5	10	5	7	69
6	0	0	0	0	0	0	0	0	0	0	0
7	5	10	5	5	10	7	9	6	6	7	70
Total	17	18	16	19	20	17	18	20	17	18	180

800 mg NaCl/L

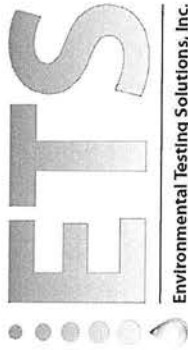
Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	5	6	5	5	4	5	5	6	5	50
5	12	11	12	11	12	10	12	12	12	13	117
6	0	0	0	0	0	0	0	0	0	0	0
7	14	15	14	17	17	17	13	17	16	17	157
Total	30	31	32	33	34	31	30	34	34	35	324

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	2	1	2	2	1	1	1	3	18
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	3	2	2	1	2	2	1	1	1	3	18

Prepared and Entered by Jim Sumner





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

Environmental Testing Solutions, Inc.

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

Test number: **CdNaClCR #282**
 Test dates: **March 07-14, 2023**

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	36	32	33	35	32	34	34	32	33	36	100	33.7	4.6	Not applicable
600	36	34	34	36	34	31	34	36	33	37	100	34.5	5.2	-2.4
800	30	31	32	33	34	31	30	34	34	35	100	32.4	5.7	
1000	29	30	29	33	28	32	32	32	34	29	100	30.8	6.6	8.6
1200	17	18	16	19	20	17	18	20	17	18	100	18.0	7.4	46.6
1400	3	2	2	1	2	2	1	1	1	3	100	1.8	43.8	94.7

Dunnett's MSD value: **1.648**
 PMSD: **4.9**

MSD = Minimum Significant Difference
 PMSD = Percent Minimum Significant Difference

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

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

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

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

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



Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date: 3/7/2023 Test ID: CdNaClCR Sample ID: REF-Ref Toxicant
 End Date: 3/14/2023 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:

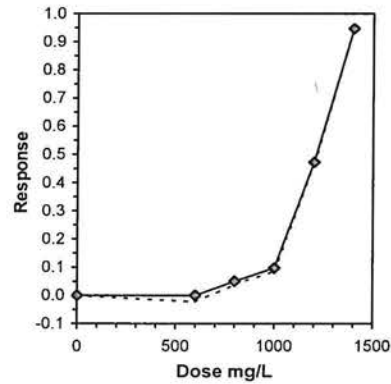
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	36.000	32.000	33.000	35.000	32.000	34.000	34.000	32.000	33.000	36.000
600	36.000	34.000	34.000	36.000	34.000	31.000	34.000	36.000	33.000	37.000
800	30.000	31.000	32.000	33.000	34.000	31.000	30.000	34.000	34.000	35.000
1000	29.000	30.000	29.000	33.000	28.000	32.000	32.000	32.000	34.000	29.000
1200	17.000	18.000	16.000	19.000	20.000	17.000	18.000	20.000	17.000	18.000
1400	3.000	2.000	2.000	1.000	2.000	2.000	1.000	1.000	1.000	3.000

Conc-mg/L	Transform: Untransformed								t-Stat	1-Tailed Critical	MSD	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean				N-Mean	
D-Control	33.700	1.0000	33.700	32.000	36.000	4.650	10				34.100	1.0000	
600	34.500	1.0237	34.500	31.000	37.000	5.158	10	-1.110	2.287	1.648	34.100	1.0000	
800	32.400	0.9614	32.400	30.000	35.000	5.672	10	1.804	2.287	1.648	32.400	0.9501	
*1000	30.800	0.9139	30.800	28.000	34.000	6.636	10	4.024	2.287	1.648	30.800	0.9032	
*1200	18.000	0.5341	18.000	16.000	20.000	7.407	10	21.788	2.287	1.648	18.000	0.5279	
*1400	1.800	0.0534	1.800	1.000	3.000	43.823	10	44.269	2.287	1.648	1.800	0.0528	

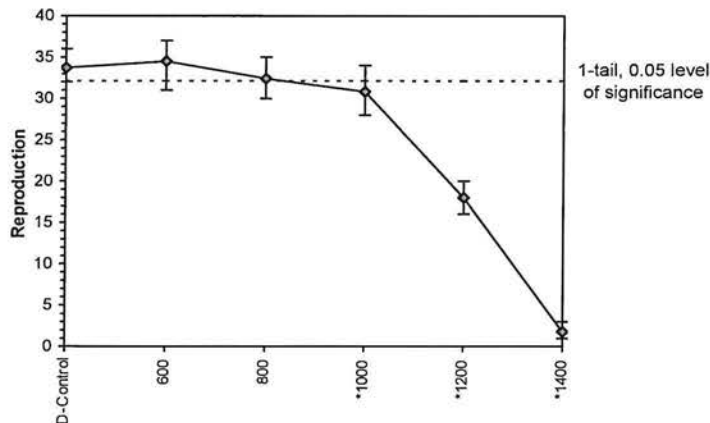
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Kolmogorov D Test indicates normal distribution ($p > 0.01$)	0.90369	1.035	0.01493	-0.7688						
Bartlett's Test indicates equal variances ($p = 0.16$)	7.98518	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	800	1000	894.427		1.64776	0.0489	1682.68	2.5963	0.0E+00	5, 54
Treatments vs D-Control										

Point	Linear Interpolation (200 Resamples)				
	mg/L	SD	95% CL		Skew
IC05	800.625	54.7671	705.144	913.825	0.5756
IC10	1001.72	44.3795	839.773	1016.38	-1.9619
IC15	1028.36	8.75541	1008.16	1042.1	-0.1864
IC20	1055	7.82169	1036.97	1068	-0.1275
IC25	1081.64	7.06611	1065.83	1093.85	-0.0538
IC40	1161.56	6.44663	1150.43	1173.34	0.2819
IC50	1211.73	5.17409	1201.96	1222.16	0.1479

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



Dose-Response Plot



Entered and
Reviewed by
Jim Sumner
JS

Species: Ceriodaphnia dubia

CdNaClCR #: 282

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		W	W	W	W	W	W
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.16	8.04	8.01	8.10	8.04	7.96
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.7	7.6	7.7
	Conductivity (µmhos/cm)	290		309		300	
	Alkalinity (mg CaCO ₃ /L)	59				59	
	Hardness (mg CaCO ₃ /L)	92				92	
	Temperature (°C)	24.9	25.0	24.8	25.2	24.8	25.0
600 mg NaCl/L	pH (S.U.)	8.12	7.99	8.02	8.03	8.04	7.89
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.7	7.6	7.7
	Conductivity (µmhos/cm)	1420		1450		1390	
	Temperature (°C)	25.1	25.2	24.8	25.0	24.9	24.8
800 mg NaCl/L	pH (S.U.)	8.11	7.98	8.02	8.03	8.04	7.90
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.7	7.6	7.7
	Conductivity (µmhos/cm)	1770		1810		1730	
	Temperature (°C)	25.0	24.9	24.9	25.0	24.8	24.8
1000 mg NaCl/L	pH (S.U.)	8.10	7.98	8.01	8.04	8.03	7.89
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.6	7.6	7.7
	Conductivity (µmhos/cm)	2120		2170		2100	
	Temperature (°C)	25.2	25.2	24.8	25.1	24.8	24.8
1200 mg NaCl/L	pH (S.U.)	8.10	7.98	8.01	8.03	8.03	7.89
	Dissolved oxygen (mg/L)	7.8	8.0	7.9	7.6	7.6	7.7
	Conductivity (µmhos/cm)	2500		2560		2460	
	Temperature (°C)	25.2	25.2	24.9	25.1	24.8	24.9
1400 mg NaCl/L	pH (S.U.)	8.10	7.97	8.02	8.04	8.06	7.91
	Dissolved oxygen (mg/L)	7.9	8.0	8.0	7.4	7.7	7.7
	Conductivity (µmhos/cm)	2970		2980		2810	
	Temperature (°C)	25.0	25.0	24.9	25.1	24.8	24.8
		Initial	Final	Initial	Final	Initial	Final

Species: Ceriodaphnia dubia

CdNaClCR #: 282

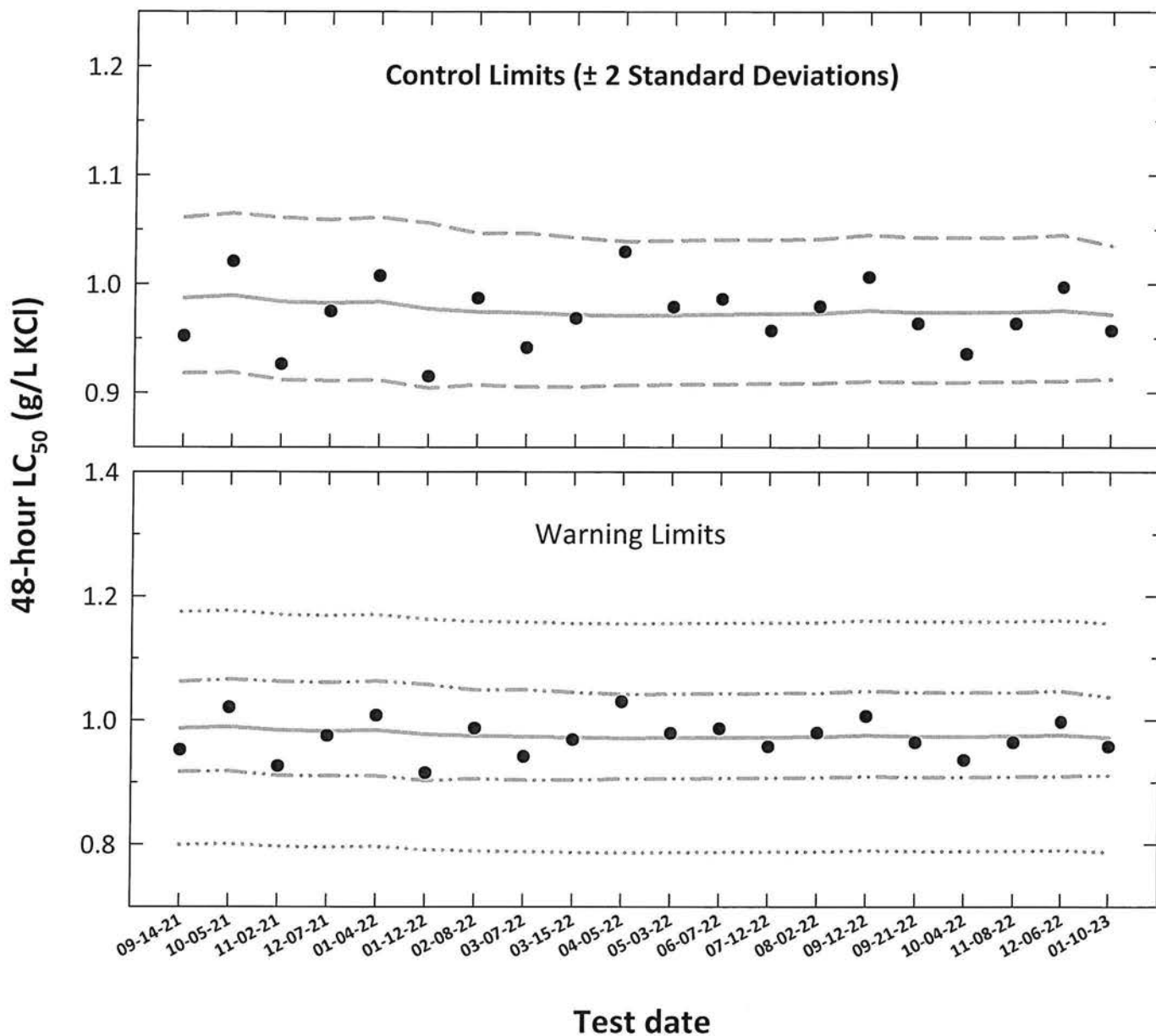
Concentration		Parameter		Day							
				(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
				3		4		5		6	
Analyst		U	BSC	BSC	BSC	BSC	BL U	BL U	U		
CONTROL, MHSW	pH (S.U.)	7.92	8.17	8.14	8.11	8.07	8.11	8.04	7.96		
	Dissolved oxygen (mg/L)	7.6	8.0	7.8	8.0	7.9	8.0	7.9	7.9		
	Conductivity (µmhos/cm)	301		299		299		301			
	Alkalinity (mg CaCO ₃ /L)			62							
	Hardness (mg CaCO ₃ /L)			91							
	Temperature (°C)	24.8	25.1	24.7	25.2	24.8	25.2	24.7	25.2		
600 mg NaCl/L	pH (S.U.)	7.97	8.14	8.16	8.08	8.09	8.06	8.12	7.96		
	Dissolved oxygen (mg/L)	7.4	8.0	7.9	8.0	* 8.45 (7.8)	7.9	7.9	7.9		
	Conductivity (µmhos/cm)	1430		1360		1420		1440			
	Temperature (°C)	24.9	24.9	24.7	25.2	24.9	25.0	24.8	25.0		
800 mg NaCl/L	pH (S.U.)	7.98	8.14	8.16	8.07	8.09	8.05	8.12	7.95		
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	8.0	7.9	8.0	8.0	8.0		
	Conductivity (µmhos/cm)	1810		1730		* (1800) 1400		1850			
	Temperature (°C)	24.8	24.9	24.7	25.0	24.8	25.2	24.8	25.0		
1000 mg NaCl/L	pH (S.U.)	7.97	8.13	8.16	8.06	8.10	8.06	8.13	7.94		
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	8.0	8.0	8.0	8.0	8.0		
	Conductivity (µmhos/cm)	2150		2080		* (2140) 1380		2220			
	Temperature (°C)	24.9	24.9	24.7	25.0	24.8	25.1	24.8	25.1		
1200 mg NaCl/L	pH (S.U.)	7.97	8.11	8.15	8.04	8.08	8.06	8.12	7.94		
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	8.0	8.0	8.0	8.0	7.9		
	Conductivity (µmhos/cm)	2540		2460		* (2510) 1650		2580			
	Temperature (°C)	24.9	25.2	24.8	25.0	24.8	25.1	24.9	24.9		
1400 mg NaCl/L	pH (S.U.)	7.98	8.11	8.16	8.03	8.08	8.04	8.13	7.94		
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	8.0	8.0	8.1	8.1	8.0		
	Conductivity (µmhos/cm)	2910		2870		* (2910) 1930		3090			
	Temperature (°C)	24.9	24.9	24.8	24.9	25.0	25.1	24.9	24.9		
		Initial	Final	Initial	Final	Initial	Final	Initial	Final		

* wrote in wrong book BSC 03.12.23

Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture



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

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

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV		
						CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - S _{A,75}	CT + S _{A,75}	Warning Limits	Warning Limits
1	09-14-21	0.9522	-0.0213	-0.0057	0.0157	0.9871	1.0613	0.9171	1.0623	0.7995	1.1746		
2	10-05-21	1.0207	0.0089	-0.0047	0.0160	0.9893	1.0650	0.9182	1.0659	0.8014	1.1773		
3	11-02-21	0.9262	-0.0333	-0.0071	0.0164	0.9837	1.0610	0.9108	1.0623	0.7968	1.1706		
4	12-07-21	0.9747	-0.0111	-0.0078	0.0163	0.9823	1.0590	0.9099	1.0604	0.7957	1.1689		
5	01-04-22	1.0075	0.0033	-0.0072	0.0165	0.9836	1.0614	0.9104	1.0626	0.7967	1.1705		
6	01-12-22	0.9151	-0.0385	-0.0100	0.0168	0.9773	1.0560	0.9027	1.0579	0.7916	1.1630		
7	02-08-22	0.9869	-0.0057	-0.0112	0.0155	0.9745	1.0465	0.9058	1.0484	0.7894	1.1597		
8	03-07-22	0.9416	-0.0261	-0.0116	0.0157	0.9737	1.0469	0.9038	1.0489	0.7887	1.1587		
9	03-15-22	0.9685	-0.0139	-0.0124	0.0153	0.9718	1.0429	0.9036	1.0450	0.7871	1.1564		
10	04-05-22	1.0297	0.0127	-0.0127	0.0147	0.9711	1.0393	0.9055	1.0414	0.7866	1.1556		
11	05-03-22	0.9788	-0.0093	-0.0125	0.0148	0.9716	1.0400	0.9059	1.0420	0.7870	1.1562		
12	06-07-22	0.9862	-0.0061	-0.0122	0.0148	0.9723	1.0410	0.9062	1.0430	0.7875	1.1570		
13	07-12-22	0.9572	-0.0190	-0.0121	0.0147	0.9726	1.0409	0.9069	1.0429	0.7878	1.1574		
14	08-02-22	0.9794	-0.0090	-0.0119	0.0148	0.9730	1.0414	0.9073	1.0433	0.7881	1.1578		
15	09-12-22	1.0063	0.0027	-0.0107	0.0149	0.9756	1.0452	0.9091	1.0469	0.7903	1.1610		
16	09-21-22	0.9638	-0.0160	-0.0114	0.0148	0.9740	1.0428	0.9080	1.0446	0.7889	1.1591		
17	10-04-22	0.9358	-0.0288	-0.0114	0.0148	0.9740	1.0427	0.9081	1.0446	0.7890	1.1591		
18	11-08-22	0.9637	-0.0160	-0.0113	0.0147	0.9744	1.0428	0.9088	1.0446	0.7892	1.1595		
19	12-06-22	0.9971	-0.0013	-0.0106	0.0149	0.9758	1.0450	0.9095	1.0468	0.7904	1.1612		
20	01-10-23	0.9572	-0.0190	-0.0123	0.0137	0.9720	1.0352	0.9109	1.0370	0.7873	1.1566		

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

S = Standard deviation of the LC₅₀ values.

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

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

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

CV = Coefficient of variation.

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

***Pimephales promelas* Potassium Chloride Acute Reference Toxicant Test**

PpKCIAC # 145

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 #: 2166

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	8.01	7.85	7.98
	Dissolved oxygen (mg/L)	7.7	7.0	7.9
	Conductivity (µmhos/cm)	3060		
	Alkalinity (mg/L CaCO ₃)	63		
	Hardness (mg/L CaCO ₃)	86		
	Temperature (°C)	24.8	24.6	24.7
500 mg/L	pH (S.U.)	8.00	7.85	7.80
	Dissolved oxygen (mg/L)	7.7	7.0	7.9
	Conductivity (µmhos/cm)	1220		
	Temperature (°C)	24.1	24.7	24.8
750 mg/L	pH (S.U.)	8.05	7.85	7.80
	Dissolved oxygen (mg/L)	7.7	7.0	7.9
	Conductivity (µmhos/cm)	11030		
	Temperature (°C)	24.7	24.7	24.8
1000 mg/L	pH (S.U.)	8.05	7.85	7.77
	Dissolved oxygen (mg/L)	7.7	7.0	7.9
	Conductivity (µmhos/cm)	2080		
	Temperature (°C)	24.9	24.5	24.6
1250 mg/L	pH (S.U.)	8.04	7.85	7.75
	Dissolved oxygen (mg/L)	7.8	7.8	8.0
	Conductivity (µmhos/cm)	2880		
	Temperature (°C)	24.9	24.8	24.6
1500 mg/L	pH (S.U.)	8.04	7.80	
	Dissolved oxygen (mg/L)	7.8	7.0	
	Conductivity (µmhos/cm)	3020		
	Temperature (°C)	24.9	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	180104324
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 # 145

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	01-10-23	* 0500 + 0945	JL	1155	JL	IF	PINK	01-04-23B
24	01-11-23			1200	JL			
48 Termination	01-12-23			1156	JL			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	12-29-22
Age (1 to 14 days old):	5 TO 6 DAYS
Hatch date and times:	01-04-23 1225 TO 01-05-23 0615
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.27
	Temperature (°C): 24.6

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	9 ^{ud}	7 ^{3d}	7 ^{3d}	2 ^{id}	1 ^{9d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9 ^{id}	9	5 ^{2u}	4 ^{3d}	0 ^{2d}	1	0	0
Mean Survival	100%		100%		90%		45%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	883.8
Upper 95% confidence limit (mg KCl/L)	1026.7
48-hour LC ₅₀ (mg KCl/L)	957.2

Comments:

Test Reviewed by: JL

Acute Fathead Minnow Test-24 Hr Survival

Start Date: 1/10/2023	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 1/12/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: ACUTE-EPA-821-R-02-012	Test Species: PP-Pimephales promelas

Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	0.9000
1000	0.7000	0.7000
1250	0.2000	0.1000
1500	0.0000	0.0000

Conc-mg/L	Transform: Arcsin Square Root							N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2					0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.1765		0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.306	2.830	0.1765		1	20
*1000	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	2	6.747	2.830	0.1765		6	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	16.341	2.830	0.1765		17	20
*1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	20.091	2.830	0.1765		20	20

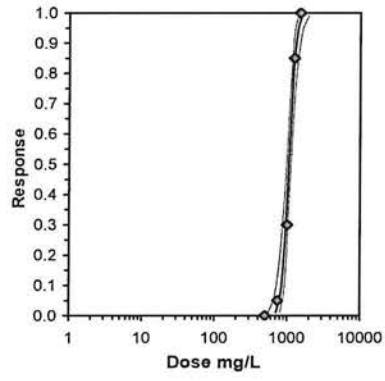
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	750	1000	866.025		0.08328	0.08542	0.60401	0.00389	2.9E-06	5, 6

Treatments vs D-Control

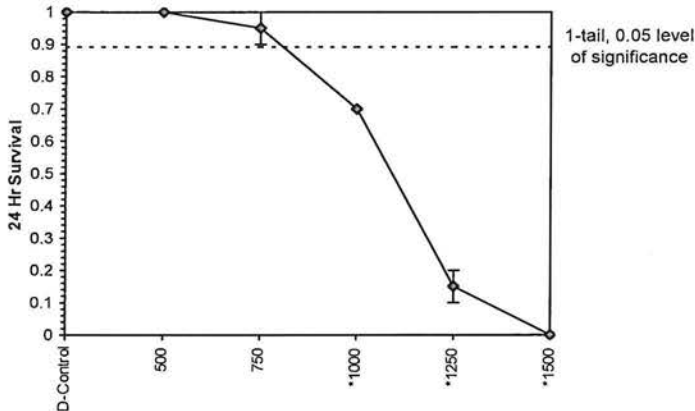
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
			Lower	Upper							
Slope	13.7115	2.52759	8.7574	18.6656	0	1.65698	7.81472	0.64654	3.0229	0.07293	3
Intercept	-36.448	7.67563	-51.493	-21.404							

TSCR

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	713.234	554.855	808.131
EC05	3.355	799.714	660.787	882.937
EC10	3.718	850.023	724.317	926.854
EC15	3.964	885.743	769.898	958.581
EC20	4.158	915.199	807.55	985.315
EC25	4.326	941.25	840.71	1009.57
EC40	4.747	1010.23	926.37	1078.08
EC50	5.000	1054.14	977.797	1126.39
EC60	5.253	1099.95	1027.72	1181.85
EC75	5.674	1180.56	1105.94	1292.3
EC80	5.842	1214.16	1135.54	1342.54
EC85	6.036	1254.54	1169.41	1405.55
EC90	6.282	1307.26	1211.5	1491.46
EC95	6.645	1389.5	1273.85	1632.17
EC99	7.326	1557.98	1393.98	1940.7



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 1/10/2023 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 1/12/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: PP-Pimephales promelas

Comments:

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

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.1565	0	20	
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.947	2.830	0.1565	2	20	
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	12.242	2.830	0.1565	11	20	
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	21.189	2.830	0.1565	19	20	
*1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	22.663	2.830	0.1565	20	20	

Auxiliary Tests Statistic Critical Skew Kurt

Normality of the data set cannot be confirmed

Equality of variance cannot be confirmed

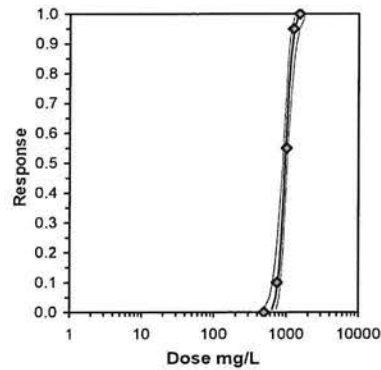
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test	500	750	612.372		0.07115	0.07297	0.66073	0.00306	1.1E-06	5, 6

Treatments vs D-Control

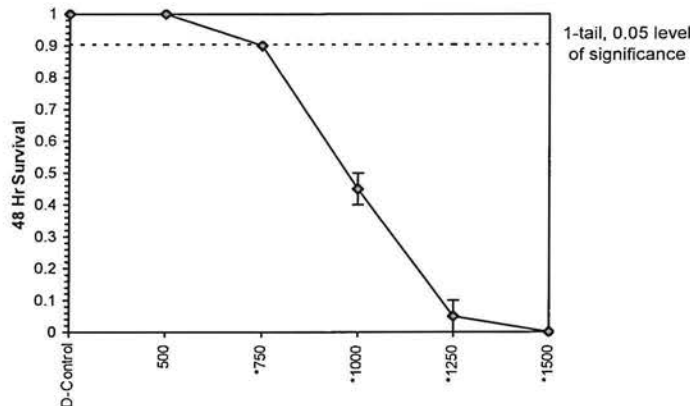
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.2642	2.42918	8.50303	18.0254	0	0.46201	7.81472	0.92715	2.98101	0.07539	3
Intercept	-34.541	7.27877	-48.807	-20.274							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	639.176	493.334	727.802
EC05	3.355	719.447	590.487	797.807
EC10	3.718	766.282	648.953	839.028
EC15	3.964	799.591	690.984	868.855
EC20	4.158	827.094	725.754	894.014
EC25	4.326	851.442	756.417	916.854
EC40	4.747	916.021	835.854	981.348
EC50	5.000	957.206	883.777	1026.74
EC60	5.253	1000.24	930.544	1078.74
EC75	5.674	1076.11	1004.31	1182.16
EC80	5.842	1107.79	1032.35	1229.27
EC85	6.036	1145.89	1064.47	1288.43
EC90	6.282	1195.7	1104.43	1369.24
EC95	6.645	1273.54	1163.66	1502.01
EC99	7.326	1433.48	1277.92	1794.53



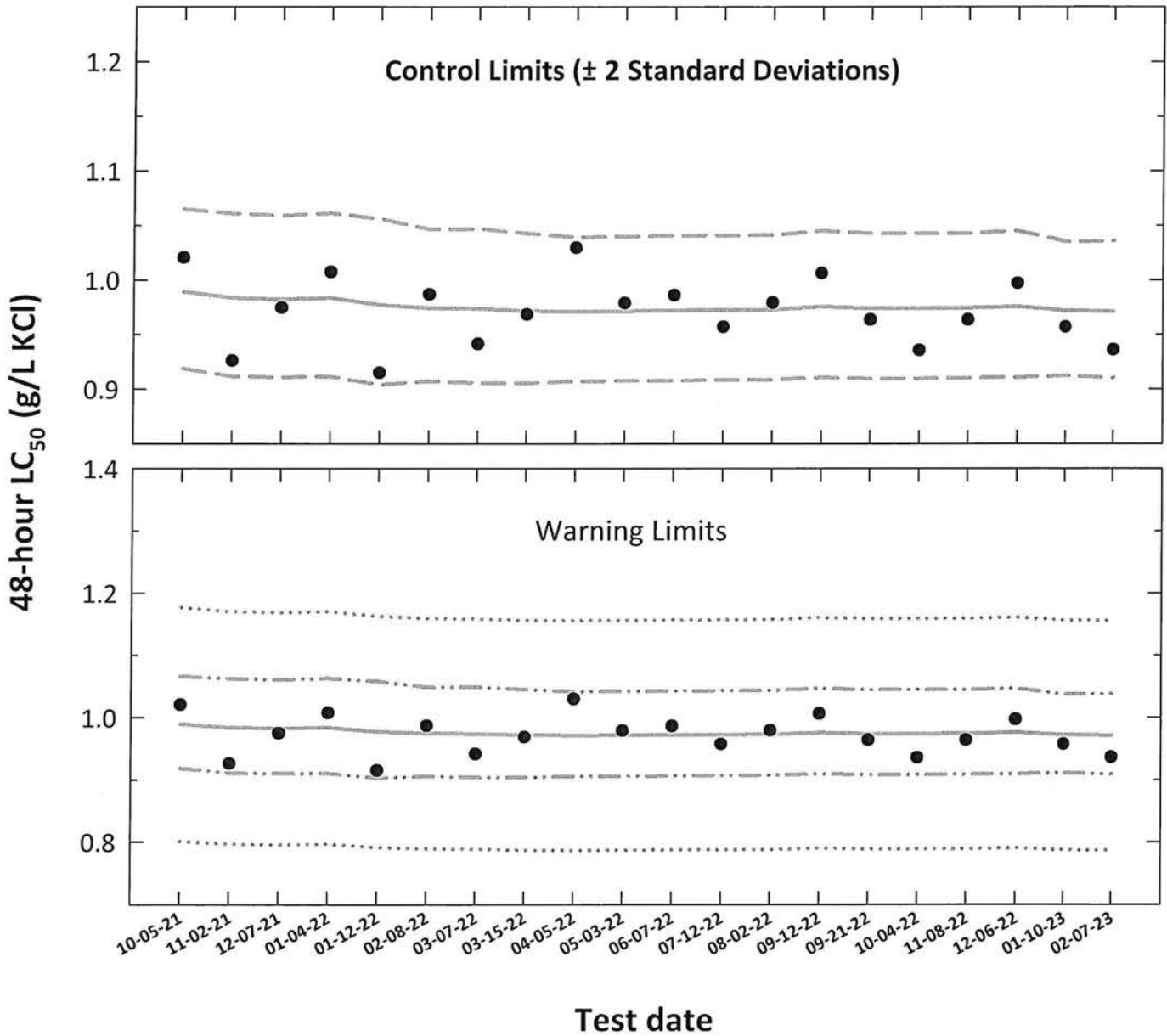
Dose-Response Plot



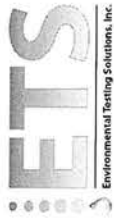
Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture



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



Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits CT - 2S CT + 2S	Laboratory Calculated CV Warning Limits CT - 2CV CT + 2CV	75th Percentile CV Warning Limits CT - S _{A,75} CT + S _{A,75}			
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	06-07-22	0.9862	-0.0061	-0.0122	0.0148	0.9723	0.9081	1.0410	0.9062	1.0430	0.7875	1.1570
12	07-12-22	0.9572	-0.0190	-0.0121	0.0147	0.9726	0.9087	1.0409	0.9069	1.0429	0.7878	1.1574
13	08-02-22	0.9794	-0.0090	-0.0119	0.0148	0.9730	0.9090	1.0414	0.9073	1.0433	0.7881	1.1578
14	09-12-22	1.0063	0.0027	-0.0107	0.0149	0.9756	0.9107	1.0452	0.9091	1.0469	0.7903	1.1610
15	09-21-22	0.9638	-0.0160	-0.0114	0.0148	0.9740	0.9097	1.0428	0.9080	1.0446	0.7889	1.1591
16	10-04-22	0.9358	-0.0288	-0.0114	0.0148	0.9740	0.9099	1.0427	0.9081	1.0446	0.7890	1.1591
17	11-08-22	0.9637	-0.0160	-0.0113	0.0147	0.9744	0.9104	1.0428	0.9088	1.0446	0.7892	1.1595
18	12-06-22	0.9971	-0.0013	-0.0106	0.0149	0.9758	0.9111	1.0450	0.9095	1.0468	0.7904	1.1612
19	01-10-23	0.9572	-0.0190	-0.0123	0.0137	0.9720	0.9126	1.0352	0.9109	1.0370	0.7873	1.1566
20	02-07-23	0.9362	-0.0286	-0.0127	0.0140	0.9711	0.9103	1.0360	0.9085	1.0379	0.7866	1.1557

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 # 146

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 #: 2166

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW		N	N	N
	pH (S.U.)	7.38	7.43	7.64
	Dissolved oxygen (mg/L)	7.6	7.9	7.8
	Conductivity (µmhos/cm)	293		
	Alkalinity (mg/L CaCO ₃)	60		
	Hardness (mg/L CaCO ₃)	86		
Temperature (°C)		24.8	24.8	24.5
500 mg/L	pH (S.U.)	7.79	7.43	7.46
	Dissolved oxygen (mg/L)	7.8	7.9	7.9
	Conductivity (µmhos/cm)	1160		
	Temperature (°C)	24.9	24.6	24.8
750 mg/L	pH (S.U.)	7.70	7.47	7.51
	Dissolved oxygen (mg/L)	7.8	7.9	7.8
	Conductivity (µmhos/cm)	1570		
	Temperature (°C)	24.9	24.6	24.7
1000 mg/L	pH (S.U.)	7.78	7.49	7.55
	Dissolved oxygen (mg/L)	7.8	7.9	7.8
	Conductivity (µmhos/cm)	2010		
	Temperature (°C)	24.8	24.6	24.7
1250 mg/L	pH (S.U.)	7.82	7.57	
	Dissolved oxygen (mg/L)	7.8	7.9	
	Conductivity (µmhos/cm)	2470		
	Temperature (°C)	24.8	24.6	
1500 mg/L	pH (S.U.)	7.87	7.60	
	Dissolved oxygen (mg/L)	7.9	7.9	
	Conductivity (µmhos/cm)	2850		
	Temperature (°C)	24.9	24.6	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg CaCO ₃ /L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg CaCO ₃ /L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	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 # 146

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	02-01-23	* 0505	JL	0705	JL	1B	ORANGE	02-01-23B
24	02-08-23			0700	JL			
48 Termination	02-09-23			0700	JL			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	01-26-23
Age (1 to 14 days old):	5 TO 6 DAYS
Hatch date and times:	02-01-23 1232 TO 02-02-23 0700
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.72
	Temperature (°C): 24.9

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

Survival Data (number of living organisms):

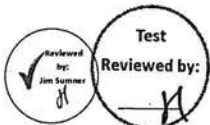
Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	9 ^{1d}	9 ^{1d}	7 ^{3d}	8 ^{2d}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	8 ^{1d}	8 ^{1d}	6 ^{1d}	5 ^{3d}	0	0	0	0
Mean Survival	100%		100%		80%		55%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	859.2
Upper 95% confidence limit (mg KCl/L)	1008.3
48-hour LC ₅₀ (mg KCl/L)	936.2

Comments:



Acute Fathead Minnow Test-24 Hr Survival

Start Date: 2/7/2023	Test ID: PpKCIAC	Sample ID: REF-Ref Toxicant
End Date: 2/9/2023	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	0.7000	0.8000
1250	0.0000	0.0000
1500	0.0000	0.0000

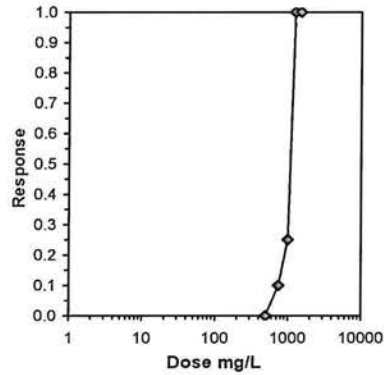
Conc-mg/L	Transform: Arcsin Square Root							N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2					0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1045		0	20
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	4.443	2.850	0.1045		2	20
*1000	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	9.893	2.850	0.1045		5	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	34.167	2.850	0.1045		20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2					20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.04275	0.04384	0.54791	0.00135	1.8E-06	4, 5

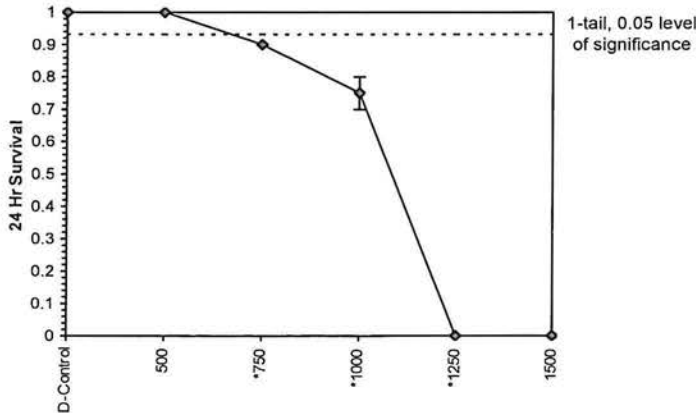
Treatments vs D-Control

Trim Level	EC50	95% CL	
0.0%	1013.15	946.652	1084.31
5.0%	1036.04	965.625	1111.59
10.0%	1052.95	985.779	1124.7
20.0%	1073.59	970.868	1187.17
Auto-0.0%	1013.15	946.652	1084.31

Trimmed Spearman-Kärber



Dose-Response Plot



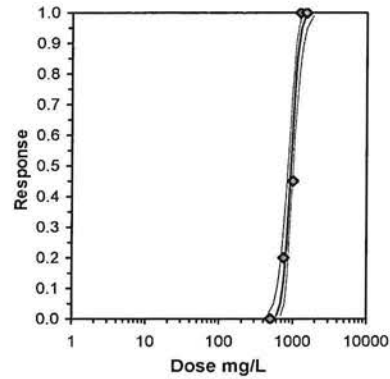
Acute Fathead Minnow Test-48 Hr Survival
 Start Date: 2/7/2023 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 2/9/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: PP-Pimephales promelas
 Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.8000	0.8000
1000	0.6000	0.5000
1250	0.0000	0.0000
1500	0.0000	0.0000

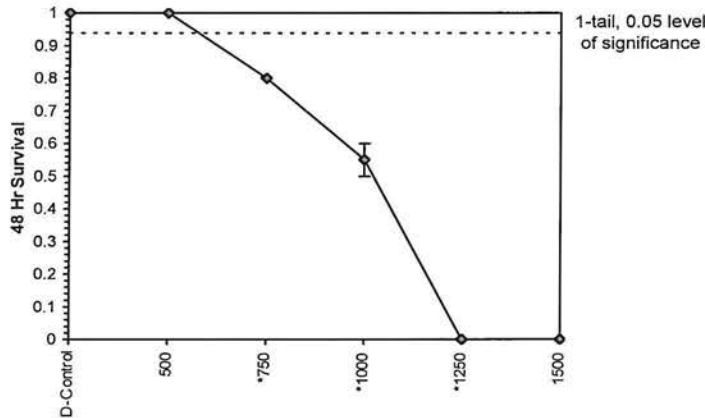
Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
*750	0.8000	0.8000	1.1071	1.1071	1.1071	0.000	2	9.576	2.850	0.0907	4	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	18.101	2.850	0.0907	9	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	39.364	2.850	0.0907	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Normality of the data set cannot be confirmed										
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.03598	0.0369	0.54226	0.00101	9.2E-07	4, 5
Treatments vs D-Control										

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	11.9436	2.15154	7.72655	16.1606	0	5.529	7.81472	0.13691	2.97135	0.08373	6
Intercept	-30.489	6.43398	-43.099	-17.878							
TSCR											
Point	Probits	mg/L	95% Fiducial Limits								
EC01	2.674	597.828	450.934	689.476							
EC05	3.355	681.766	549.775	763.518							
EC10	3.718	731.229	610.152	807.359							
EC15	3.964	766.614	653.956	839.166							
EC20	4.158	795.953	690.445	866.036							
EC25	4.326	822.018	722.813	890.453							
EC40	4.747	891.543	807.511	959.521							
EC50	5.000	936.169	859.185	1008.28							
EC60	5.253	983.029	909.978	1064.4							
EC75	5.674	1066.17	990.645	1177.02							
EC80	5.842	1101.08	1021.45	1228.73							
EC85	6.036	1143.22	1056.83	1294.01							
EC90	6.282	1198.55	1101	1383.71							
EC95	6.645	1285.5	1166.82	1532.26							
EC99	7.326	1465.99	1294.91	1864.08							



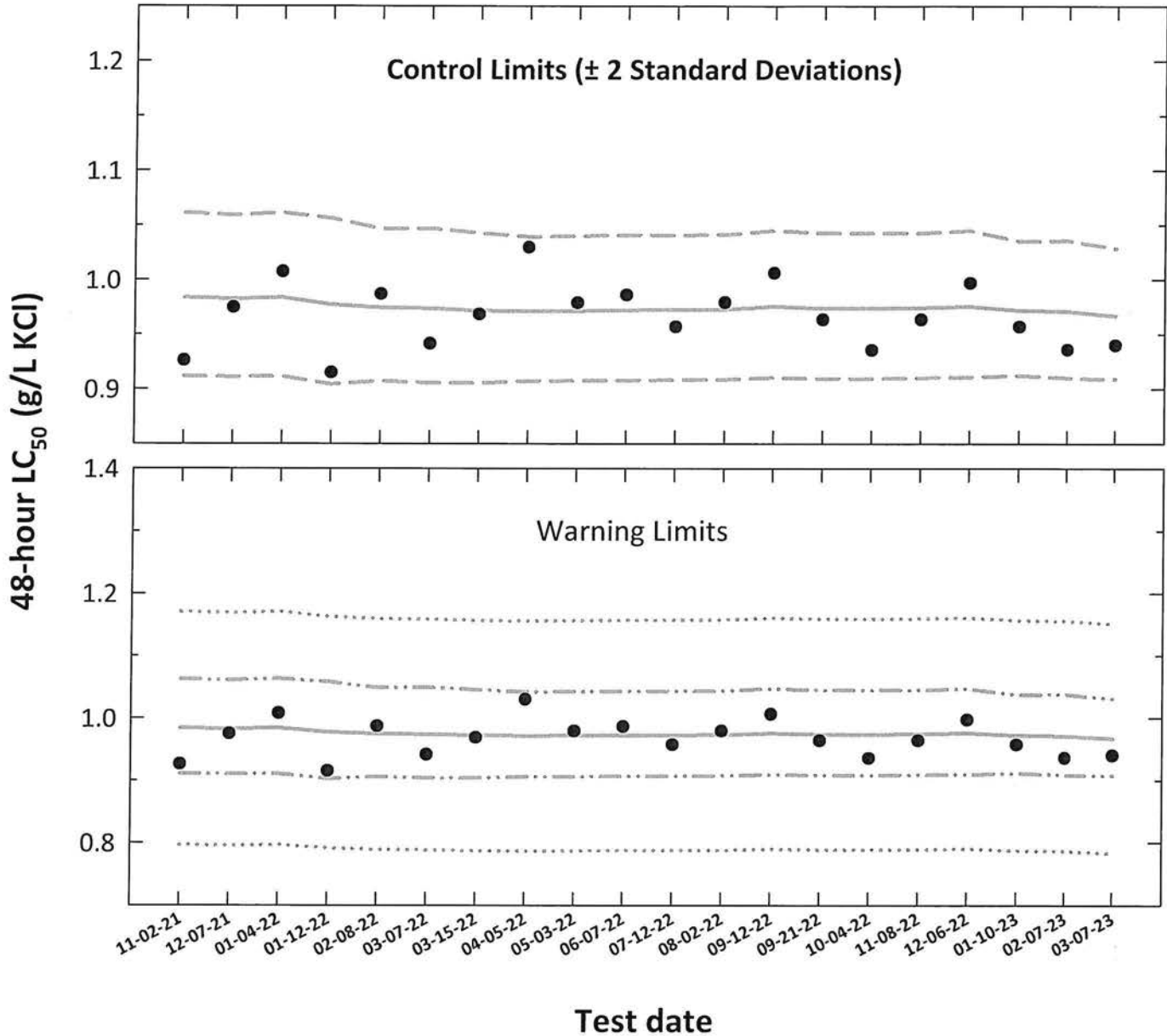
Dose-Response Plot



Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture



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

Pimephales promelas

Acute Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	48-hour LC ₅₀ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			48-hour LC ₅₀	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV		CT - S _{A,75}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	06-07-22	0.9862	-0.0061	-0.0122	0.0148	0.9723	0.9081	1.0410	0.9062	1.0430	0.7875	1.1570
11	07-12-22	0.9572	-0.0190	-0.0121	0.0147	0.9726	0.9087	1.0409	0.9069	1.0429	0.7878	1.1574
12	08-02-22	0.9794	-0.0090	-0.0119	0.0148	0.9730	0.9090	1.0414	0.9073	1.0433	0.7881	1.1578
13	09-12-22	1.0063	0.0027	-0.0107	0.0149	0.9756	0.9107	1.0452	0.9091	1.0469	0.7903	1.1610
14	09-21-22	0.9638	-0.0160	-0.0114	0.0148	0.9740	0.9097	1.0428	0.9080	1.0446	0.7889	1.1591
15	10-04-22	0.9358	-0.0288	-0.0114	0.0148	0.9740	0.9099	1.0427	0.9081	1.0446	0.7890	1.1591
16	11-08-22	0.9637	-0.0160	-0.0113	0.0147	0.9744	0.9104	1.0428	0.9088	1.0446	0.7892	1.1595
17	12-06-22	0.9971	-0.0013	-0.0106	0.0149	0.9758	0.9111	1.0450	0.9095	1.0468	0.7904	1.1612
18	01-10-23	0.9572	-0.0190	-0.0123	0.0137	0.9720	0.9126	1.0352	0.9109	1.0370	0.7873	1.1566
19	02-07-23	0.9362	-0.0286	-0.0127	0.0140	0.9711	0.9103	1.0360	0.9085	1.0379	0.7866	1.1557
20	03-07-23	0.9402	-0.0268	-0.0145	0.0134	0.9672	0.9093	1.0287	0.9073	1.0308	0.7834	1.1509

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 # 147

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 #: 2166

Chemical Analyses:

Concentration	Analyst	Hours		
		0	24	48
Control, MHSW	pH (S.U.)	8.10	8.02	7.99
	Dissolved oxygen (mg/L)	7.8	7.7	7.6
	Conductivity (µmhos/cm)	290		
	Alkalinity (mg/L CaCO ₃)	59		
	Hardness (mg/L CaCO ₃)	92		
	Temperature (°C)	24.7	24.6	24.7
500 mg/L	pH (S.U.)	8.10	7.98	7.95
	Dissolved oxygen (mg/L)	7.9	7.7	7.7
	Conductivity (µmhos/cm)	1220		
	Temperature (°C)	24.9	24.7	24.6
750 mg/L	pH (S.U.)	8.14	7.98	7.93
	Dissolved oxygen (mg/L)	7.9	7.7	7.7
	Conductivity (µmhos/cm)	1590		
	Temperature (°C)	24.9	24.7	24.6
1000 mg/L	pH (S.U.)	8.12	7.96	7.91
	Dissolved oxygen (mg/L)	7.9	7.7	7.7
	Conductivity (µmhos/cm)	2030		
	Temperature (°C)	24.8	24.5	24.6
1250 mg/L	pH (S.U.)	8.11	7.94	
	Dissolved oxygen (mg/L)	7.9	7.8	
	Conductivity (µmhos/cm)	2420		
	Temperature (°C)	24.9	24.5	
1500 mg/L	pH (S.U.)	8.10	7.93	
	Dissolved oxygen (mg/L)	8.0	7.9	
	Conductivity (µmhos/cm)	2090		
	Temperature (°C)	24.9	24.5	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on 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 # 147

Hours	Date	Feeding		Test Initiation or Termination		Location Incubator/Shelf	Randomizing Template	MHSW Batch
		Time	Analyst	Time	Analyst			
0 Initiation	03-07-13*	0504	J	0704	J	1E	PINK	02-28-13A
24	03-08-13			0700	J			
48 Termination	03-09-13			0700	J			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	02-23-13
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	02-28-13 1445 TO 03-01-13 0830
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.15
	Temperature (°C): 24.4

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 ^{1d}	6 ^{4d}	6 ^{4d}	0 ^{10d}	0 ^{10d}	0 ^{10d}	0 ^{10d}
48 Termination	10	10	10	10	9 ^{1d}	8 ^{1d}	5 ^{1d}	5 ^{1d}	0	0	0	0
Mean Survival	100%		100%		85%		50%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	67.2
Upper 95% confidence limit (mg KCl/L)	1009.2
48-hour LC ₅₀ (mg KCl/L)	940.2

Comments:



Acute Fathead Minnow Test-24 Hr Survival

Start Date: 3/7/2023 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
End Date: 3/9/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: PP-Pimephales promelas

Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	0.9000
1000	0.6000	0.6000
1250	0.0000	0.0000
1500	0.0000	0.0000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					N	t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
			Mean	Min	Max	CV%							
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20	
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1469	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	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	24.318	2.850	0.1469	20	20	
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20	

Auxiliary Tests

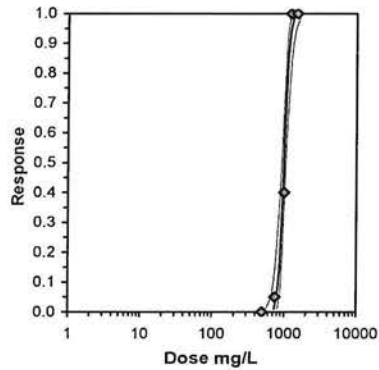
Normality of the data set cannot be confirmed
Equality of variance cannot be confirmed

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.06555	0.06723	0.58072	0.00266	8.5E-06	4, 5

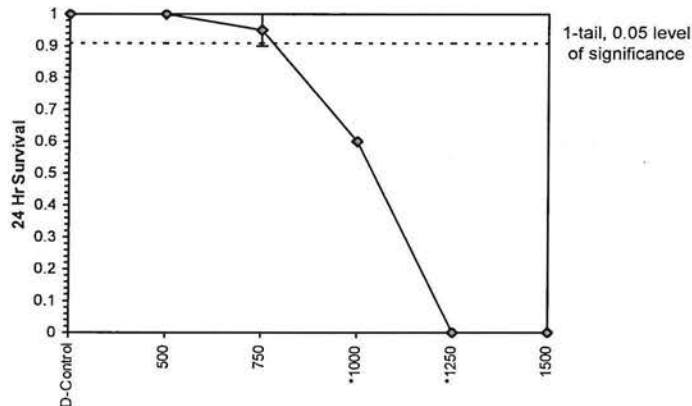
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.1625	3.55509	10.1945 24.1304	0	3.22985	7.81472	0.35753	2.99782	0.05827	6
Intercept	-46.45	10.6997	-67.421 -25.479							

Point	Probits	mg/L	95% Fiducial Limits
EC01	2.674	728.226	570.842 813.013
EC05	3.355	797.948	663.071 871.255
EC10	3.718	837.806	717.288 905.116
EC15	3.964	865.816	755.722 929.476
EC20	4.158	888.744	787.183 949.964
EC25	4.326	908.897	814.678 968.539
EC40	4.747	961.73	884.751 1021.05
EC50	5.000	994.982	926.087 1058.19
EC60	5.253	1029.38	965.58 1100.98
EC75	5.674	1089.22	1025.91 1186.38
EC80	5.842	1113.92	1048.21 1225.19
EC85	6.036	1143.42	1073.38 1273.75
EC90	6.282	1181.64	1104.2 1339.65
EC95	6.645	1240.67	1149.04 1446.75
EC99	7.326	1359.45	1233.37 1677.75



Dose-Response Plot



Acute Fathead Minnow Test-48 Hr Survival

Start Date: 3/7/2023 Test ID: PpKCIAC Sample ID: REF-Ref Toxicant
 End Date: 3/9/2023 Lab ID: ETS-Envir. Testing Sol. Sample Type: KCL-Potassium chloride
 Sample Date: Protocol: ACUTE-EPA-821-R-02-012 Test Species: PP-Pimephales promelas
 Comments:

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.8000
1000	0.5000	0.5000
1250	0.0000	0.0000
1500	0.0000	0.0000

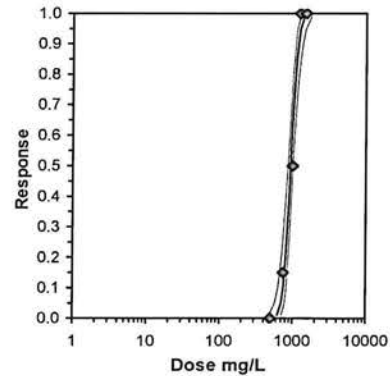
Conc-mg/L	Transform: Arcsin Square Root							t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N					
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*750	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	5.213	2.850	0.1279	3	20
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	13.965	2.850	0.1279	10	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	27.929	2.850	0.1279	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

Auxiliary Tests

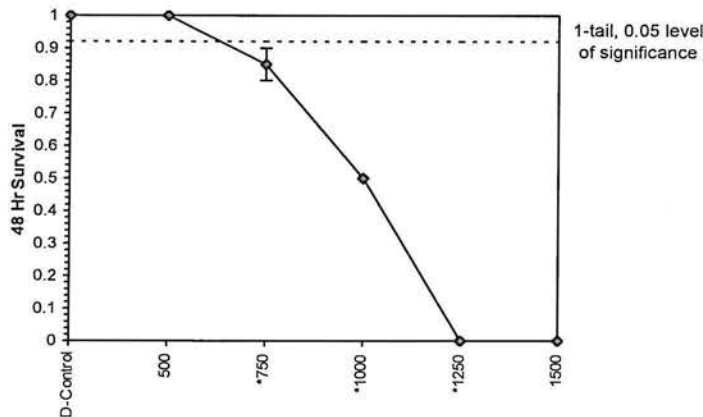
	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	500	750	612.372	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
			Lower	Upper							
Slope	13.2048	2.434	8.43416	17.9754	0	3.43755	7.81472	0.32895	2.97323	0.07573	6
Intercept	-34.261	7.2748	-48.52	-20.002							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	626.692	481.814	714.502
EC05	3.355	705.77	577.546	783.449
EC10	3.718	751.928	635.219	824.061
EC15	3.964	784.764	676.707	853.456
EC20	4.158	811.881	711.043	878.257
EC25	4.326	835.89	741.333	900.778
EC40	4.747	899.585	819.843	964.412
EC50	5.000	940.217	867.221	1009.24
EC60	5.253	982.685	913.452	1060.65
EC75	5.674	1057.57	986.335	1163.04
EC80	5.842	1088.84	1014.02	1209.73
EC85	6.036	1126.46	1045.72	1268.39
EC90	6.282	1175.66	1085.15	1348.59
EC95	6.645	1252.54	1143.57	1480.43
EC99	7.326	1410.6	1256.26	1771.28



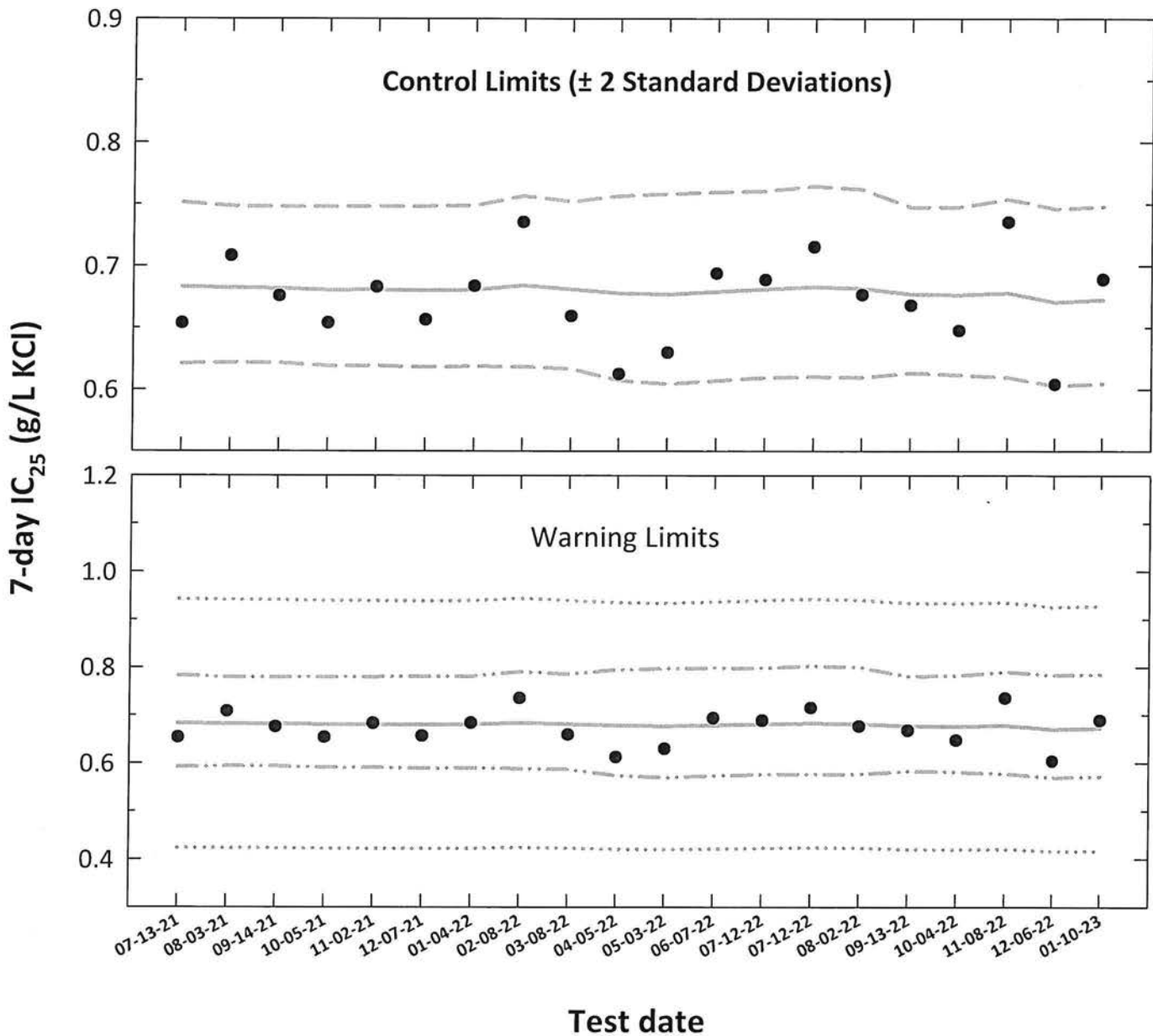
Dose-Response Plot



Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture

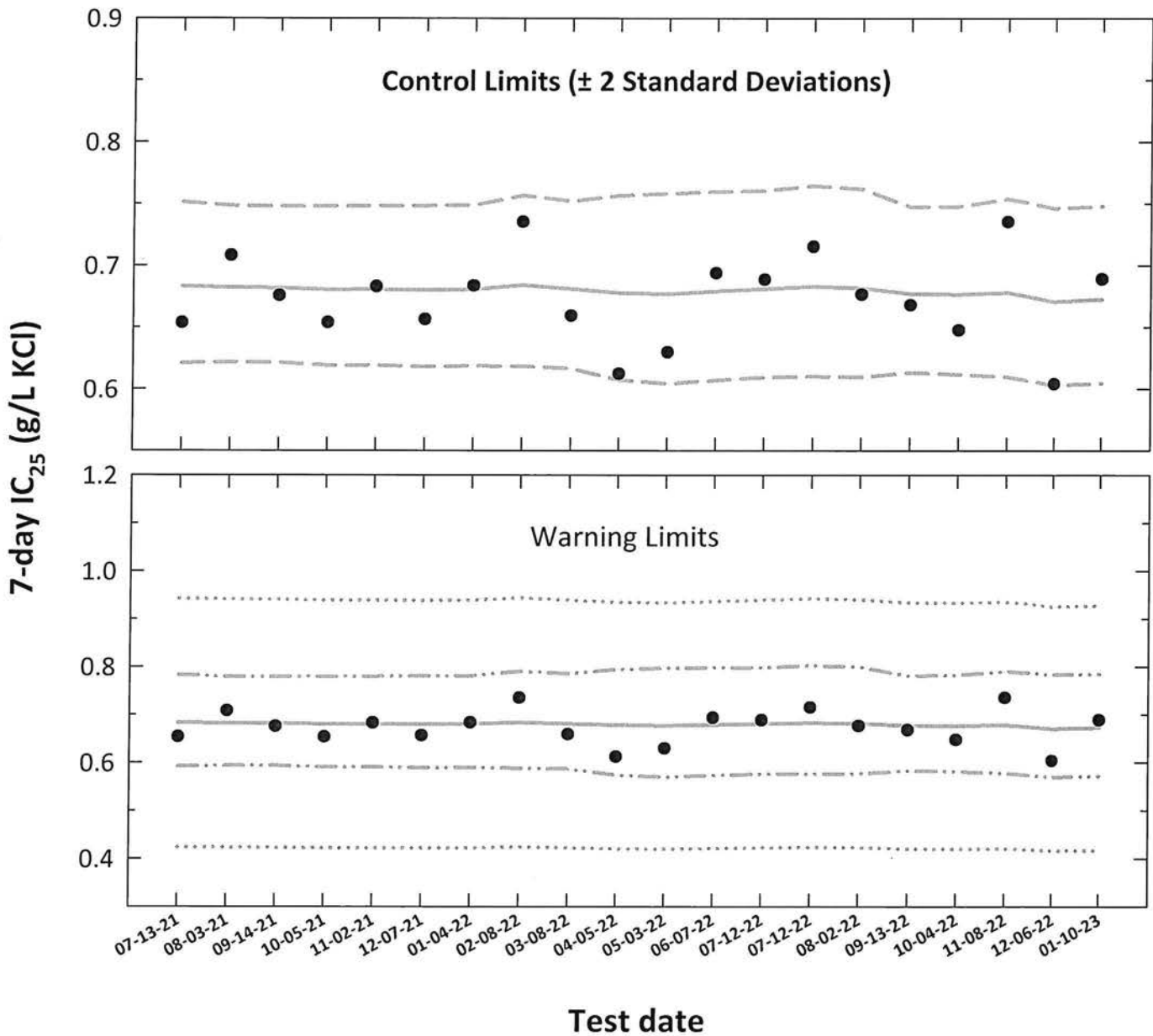


- **7-day IC₂₅** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - - **Control Limits** (mean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

Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture



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

Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							Warning Limits	Warning Limits	Warning Limits	Warning Limits		
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	07-12-22	0.6887	-0.1620	-0.1669	0.0240	0.6810	0.6096	0.7607	0.5761	0.7980	0.4222	0.9397
14	07-12-22	0.7153	-0.1455	-0.1655	0.0245	0.6831	0.6104	0.7646	0.5767	0.8023	0.4235	0.9427
15	08-02-22	0.6766	-0.1697	-0.1665	0.0242	0.6816	0.6097	0.7620	0.5762	0.7995	0.4226	0.9406
16	09-13-22	0.6682	-0.1751	-0.1693	0.0215	0.6772	0.6135	0.7475	0.5831	0.7810	0.4199	0.9345
17	10-04-22	0.6477	-0.1886	-0.1698	0.0218	0.6764	0.6118	0.7478	0.5809	0.7820	0.4194	0.9334
18	11-08-22	0.7354	-0.1335	-0.1687	0.0230	0.6781	0.6098	0.7540	0.5774	0.7900	0.4204	0.9357
19	12-06-22	0.6041	-0.2189	-0.1735	0.0232	0.6707	0.6029	0.7462	0.5695	0.7833	0.4158	0.9256
20	01-10-23	0.6890	-0.1618	-0.1722	0.0231	0.6726	0.6047	0.7481	0.5717	0.7848	0.4170	0.9282

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.

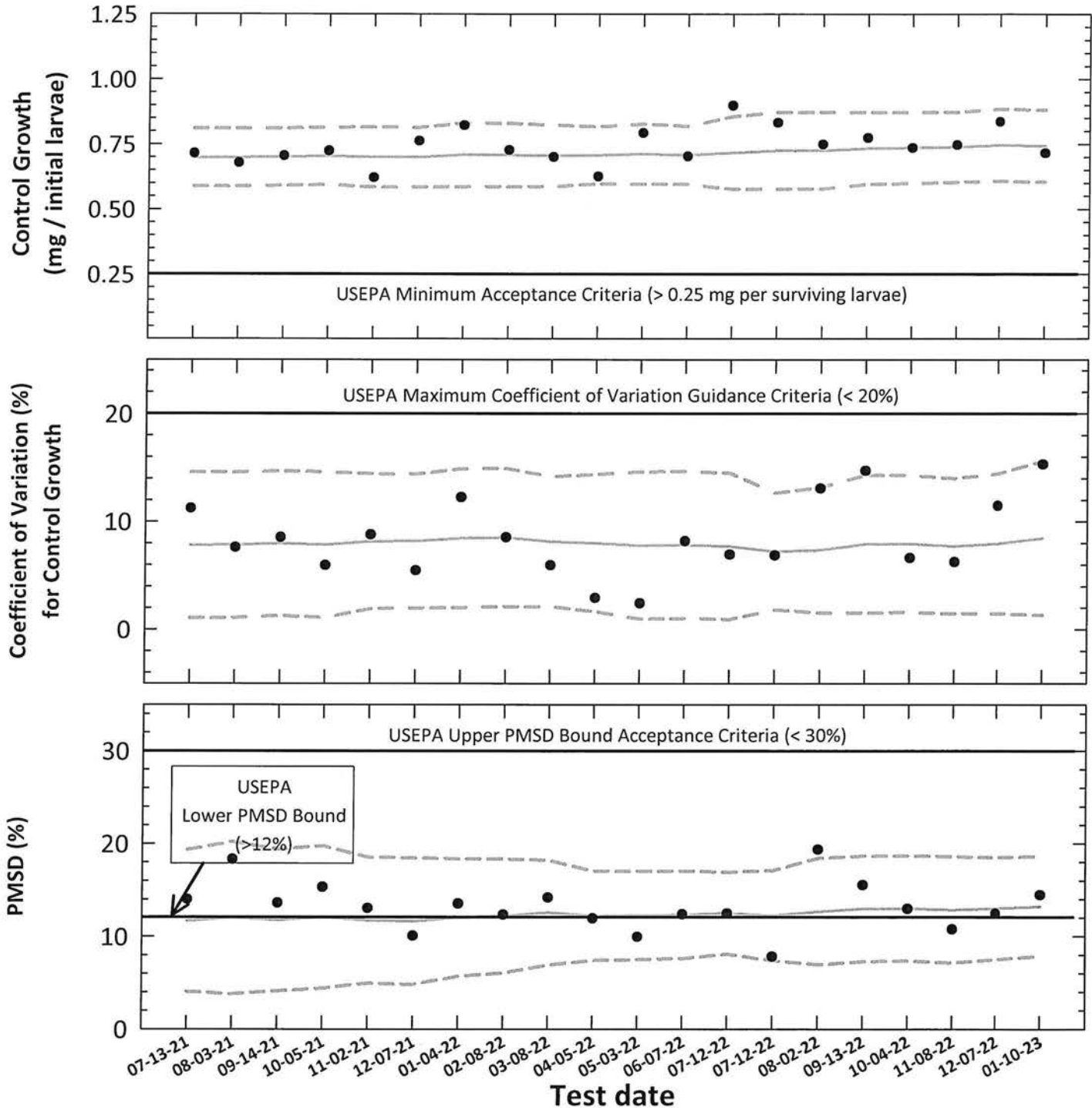
Warning Limits = Mean logarithmic IC₂₅ ± ZCV or S_{A,75} converted to anti-logarithmic values.

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)

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	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	0.810	7.8	11.7	4.0	19.3
2	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	12.0	3.8	20.2
3	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	11.7	4.1	19.4
4	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	12.1	4.4	19.7
5	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	11.7	4.9	18.5
6	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	11.6	4.8	18.5
7	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	12.0	5.7	18.3
8	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	12.2	6.0	18.3
9	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	12.6	6.9	18.2
10	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	12.2	7.4	17.0
11	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	12.3	7.5	17.0
12	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	12.3	7.6	17.0
13	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	12.5	8.1	16.9
14	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	12.2	7.4	17.1
15	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	12.7	6.9	18.4
16	09-13-22	100	0.774	14.7	0.1203	15.5	0.734	0.596	0.872	7.9	13.0	7.3	18.7
17	10-04-22	100	0.736	6.6	0.0955	13.0	0.736	0.600	0.873	7.9	13.0	7.4	18.7
18	11-08-22	100	0.747	6.3	0.0804	10.8	0.739	0.604	0.874	7.7	12.9	7.1	18.6
19	12-07-22	100	0.837	11.5	0.1043	12.5	0.747	0.608	0.886	8.0	13.0	7.5	18.5
20	01-10-23	100	0.716	15.3	0.1037	14.5	0.744	0.605	0.882	8.5	13.2	7.9	18.6

Note: Control Survival = USEPA minimum test acceptability criteria \geq 80% survival.
Control Mean Growth = USEPA minimum test acceptability criteria \geq 0.25 mg/surviving larvae.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%
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*

PpKICR Test Number: 98

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2166</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>01-03-23</u>	Artemia CHM number:	CHM1222
Hatch dates and times:	<u>01-09-23 1500 TO 01-10-23 0455</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>8.27</u> S.U. Temperature = <u>24.6</u> °C	Date / Time in oven:	<u>01-17-23 0720</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>01-18-23 0720</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	01-10-23	0500	JL	1145	JL	0700	JL	01-04-23 B
1	01-11-23	0500	JL	1100	JL	0700	JL	↓
2	01-12-23	0500	JL	1130	JL	0700	JL	01-04-23 C
3	01-13-23	0500	JL	1115	JL	0700	JL	↓
4	01-14-23	0600	JL	1200	JL	0800	JL	01-04-23 E
5	01-15-23	0550	JL	1150	JL	0750	JL	↓
6	01-16-23	0500	JL	1100	JL	0706	JL	↓
7	01-17-23					0612	JL	

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>760.5</u>
Average weight per initial larvae:	<u>0.716</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.716</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>689.0</u>

Species: Pimephales promelas

PpKCICR Test Number: 98

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 ^{15M}	10	10 ^{11F}	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>TURQUISO</u> Analyst: <u>EC</u> Date: <u>12-23-22</u>	14.27	13.17	14.05	15.28	14.97	15.55	14.78	13.82	14.02	14.41	12.72	15.29
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>01-20-23</u>	20.02	20.09	22.95	22.91	23.10	23.10	23.55	22.44	21.31	21.35	19.73	22.10
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>	5.75	6.92	8.30	7.68	8.13	8.05	8.77	8.62	7.29	6.89	7.01	7.31
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>	0.575	0.692	0.830	0.768	0.813	0.805	0.877	0.862	0.729	0.689	0.701	0.731
Average weight per initial number of larvae (mg)	0.716				0.839		-17.27		0.713		0.57	
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: 98

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	7 ^{sd}	7 ^{sd}	7 ^{sd}	7 ^{sd}
2	10	10	10	10	10	10	10	10	7	7	7	7
3	10	10	10	10	9 ^{id}	10	10	10	7	7	7	7
4	10	10	10	10	8 ^{id}	9 ^{id}	9 ^{id}	10	7	7	7	7
5	10	10	10	10	7 ^{id}	7 ^{sd}	6 ^{sd}	6 ^{sd}	2 ^{sd}	1 ^{sd}	2 ^{sd}	1 ^{sd}
6	9 ^{id}	10	10	10	6 ^{id}	7	5 ^{id}	6	1 ^{id}	1	1 ^{id}	1
7	9	9 ^{id}	10	10	6	6 ^{id}	5	6	1	1	1	1
*A = Pan weight (mg) Tray color code: <u>IVRQUAISE</u> Analyst: <u>EC</u> Date: <u>12-23-22</u>	14.33	15.72	15.48	14.37	14.45	12.68	14.04	14.94	13.52	12.47	14.30	14.07
*B = Pan + Larvae weight (mg) Analyst: <u>EC</u> Date: <u>01-20-23</u>	21.08	21.42 23.42	23.42	21.83	19.25	17.87	18.25	20.07	14.83	13.67	15.93	15.07
C = Larvae weight (mg) = B - A Analyst: <u>JA</u>	6.75	7.70	7.94	7.46	4.80	5.19	4.21	5.13	1.31	1.20	1.13	1.00
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JA</u>	0.675	0.770	0.794	0.746	0.480	0.519	0.421	0.513	0.131	0.120	0.113	0.100
Average weight per initial number of larvae (mg)	0.746		-4.27		0.483		32.57		0.116		83.87	
Percent reduction from control (%)												

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


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

Comments:

Species: Pimephales promelas

PpKCICR Test Number: 98

Survival and Growth Data

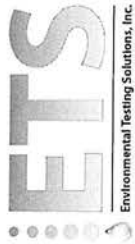
Day	1050 mg KCl/L				
	Y	Z	AA	BB	
0	10	10	10	10	
1	4 rd 6	6 rd 6	6 rd 6	5 rd 5	
2	4 rd	4 rd	6	4 rd	
3	3 rd	4	4 rd	4	
4	2 rd	4	3 rd	2 rd	
5	1 rd	1 rd	1 rd	1 rd	
6	0 rd	1	1	0 rd	
7	0	0 rd	0 rd	0	
*A = Pan weight (mg) Tray color code: <u>TURQUOISE</u> Analyst: <u>EC</u> Date: <u>12-23-22</u>		15.30	14.40	14.30	13.32
*B = Pan + Larvae weight (mg) Analyst: _____ Date: _____					
C = Larvae weight (mg) = B - A Analyst: _____					
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: _____		0	0	0	0
Average weight per initial number of larvae (mg)	Percent reduction from control (%)	0	100.		

k
01-17-23

*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: **98**
 Test dates: **January 10-17, 2023**

Concentration (mg/L KO)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight / initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.27	20.02	5.75	0.575	0.716	15.3	0.575	100.0	0.716	15.3	Not applicable
	B	10	10	13.17	20.09	6.92	0.692			0.692				
	C	10	10	14.65	22.95	8.30	0.830			0.830				
	D	10	10	15.28	22.96	7.68	0.768			0.768				
300	E	10	10	14.97	23.10	8.13	0.813	0.839	4.2	0.813	100.0	0.839	4.2	-17.2
	F	10	10	15.55	23.60	8.05	0.805			0.805				
	G	10	10	14.78	23.55	8.77	0.877			0.877				
	H	10	10	13.82	22.44	8.62	0.862			0.862				
450	I	10	10	14.02	21.31	7.29	0.729	0.713	2.9	0.729	100.0	0.713	2.9	0.5
	J	10	10	14.46	21.35	6.89	0.689			0.689				
	K	10	10	12.72	19.73	7.01	0.701			0.701				
	L	10	10	15.29	22.60	7.31	0.731			0.731				
600	M	10	9	14.33	21.08	6.75	0.750	0.786	6.5	0.675	95.0	0.746	6.9	-4.2
	N	10	9	15.72	23.42	7.70	0.856			0.770				
	O	10	10	15.48	23.42	7.94	0.794			0.794				
	P	10	10	14.37	21.83	7.46	0.746			0.746				
750	Q	10	6	14.45	19.25	4.80	0.800	0.841	3.4	0.480	57.5	0.483	9.3	32.5
	R	10	6	12.68	17.87	5.19	0.865			0.519				
	S	10	5	14.04	18.25	4.21	0.842			0.421				
	T	10	6	14.94	20.07	5.13	0.855			0.513				
900	U	10	1	13.52	14.83	1.31	1.310	1.160	11.2	0.131	10.0	0.116	11.2	83.8
	V	10	1	12.47	13.67	1.20	1.200			0.120				
	W	10	1	14.80	15.93	1.13	1.130			0.113				
	X	10	1	14.67	15.67	1.00	1.000			0.100				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.000	0.0	0.000	0.0	0.000	0.0	100.0
	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: $\frac{0.1037}{14.5}$
 PMSD: Minimum Significant Difference
 Percent Minimum Significant Difference

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



Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 1/10/2023	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant	
End Date: 1/17/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride	
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas	

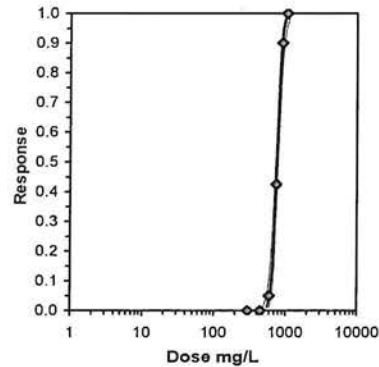
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	0.9000	0.9000	1.0000	1.0000
750	0.6000	0.6000	0.5000	0.6000
900	0.1000	0.1000	0.1000	0.1000
1050	0.0000	0.0000	0.0000	0.0000

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	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.5750	0.5750	0.8609	0.7854	0.8861	5.847	4	10.00	10.00	17	40
*900	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	4	10.00	10.00	36	40
1050	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

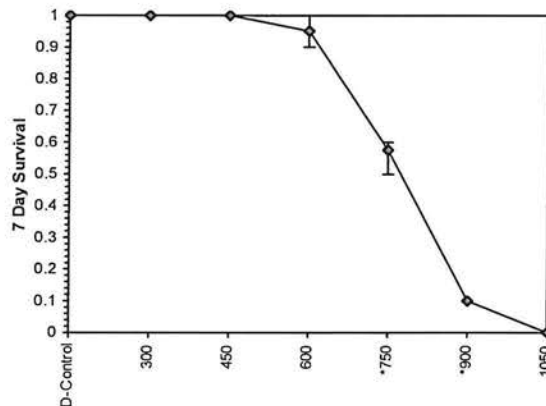
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.7435	0.884	-0.3171	1.94157
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	17.4675	2.27934	13	21.935	0	0.69047	9.48773	0.9525	2.88108	0.05725	3
Intercept	-45.325	6.58808	-58.238	-32.413							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	559.632	496.321	602.835
EC05	3.355	612.235	558.531	649.24
EC10	3.718	642.268	594.358	675.949
EC15	3.964	663.36	619.5	694.946
EC20	4.158	680.616	639.965	710.726
EC25	4.326	695.778	657.799	724.839
EC40	4.747	735.496	703.322	763.441
EC50	5.000	760.474	730.578	789.408
EC60	5.253	786.3	757.306	817.964
EC75	5.674	831.186	800.258	871.708
EC80	5.842	849.702	816.901	895.173
EC85	6.036	871.805	836.155	923.967
EC90	6.282	900.434	860.325	962.301
EC95	6.645	944.606	896.402	1023.24
EC99	7.326	1033.39	966.138	1150.63



Dose-Response Plot



Entered and Reviewed by
 Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

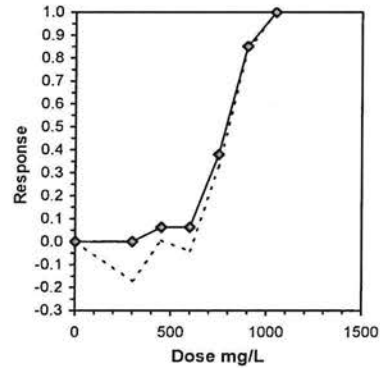
Start Date: 1/10/2023	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 1/17/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas

Conc-mg/L	1	2	3	4
D-Control	0.5750	0.6920	0.8300	0.7680
300	0.8130	0.8050	0.8770	0.8620
450	0.7290	0.6890	0.7010	0.7310
600	0.6750	0.7700	0.7940	0.7460
750	0.4800	0.5190	0.4210	0.5130
900	0.1310	0.1200	0.1130	0.1000
1050	0.0000	0.0000	0.0000	0.0000

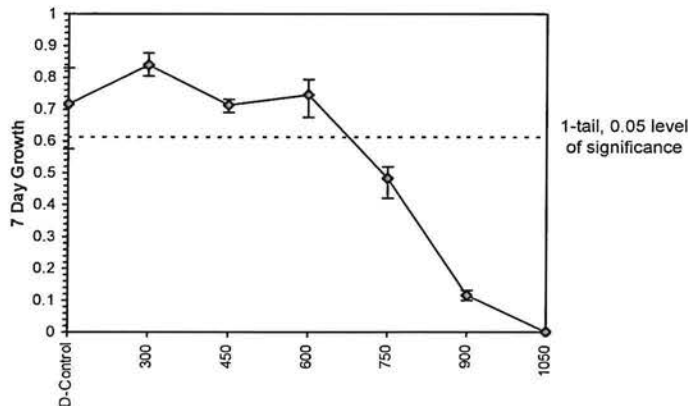
Conc-mg/L	Transform: Untransformed							1-Tailed		Isotonic		
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7163	1.0000	0.7163	0.5750	0.8300	15.327	4				0.7778	1.0000
300	0.8393	1.1717	0.8393	0.8050	0.8770	4.243	4	-2.717	2.290	0.1037	0.7778	1.0000
450	0.7125	0.9948	0.7125	0.6890	0.7310	2.921	4	0.083	2.290	0.1037	0.7294	0.9378
600	0.7463	1.0419	0.7463	0.6750	0.7940	6.886	4	-0.663	2.290	0.1037	0.7294	0.9378
750	0.4833	0.6747	0.4833	0.4210	0.5190	9.292	4				0.4833	0.6213
900	0.1160	0.1620	0.1160	0.1000	0.1310	11.196	4				0.1160	0.1491
1050	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95022	0.844	-0.5916	1.93504
Bartlett's Test indicates equal variances (p = 0.06)	7.32735	11.3449		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	600	>600		
Treatments vs D-Control	0.10366	0.14473	0.01397	0.0041
			0.05319	3, 12

Point	Linear Interpolation (200 Resamples)				
	mg/L	SD	95% CL(Exp)	Skew	
IC05	420.58	103.22	335.16	744.16	0.3676
IC10	617.92	53.16	324.27	663.51	-2.6244
IC15	641.62	13.64	600.76	687.93	0.1065
IC20	665.32	13.48	630.00	712.76	0.1959
IC25	689.02	13.73	654.08	737.57	0.2481
IC40	756.78	10.89	719.15	786.43	-0.3740
IC50	788.55	8.23	762.45	812.77	-0.2137



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

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

Test type: P. promelas Chronic Reference Toxicant Test
 Test dates: January 10-17, 2023
 Associated test: PpKCICR # 98

1st Weight = Pans were dried for 24-hours at 60 ± 2°C, desiccated, and weighed following standard operating procedures.

2nd Weight = The same pans used for determining the 1st weight measurements were dried for an additional 24-hours at 60 ± 2°C, desiccated, and weighed following standard operating procedures.

	1st Weight	2nd Weight	Difference (mg)	Percent Difference from 1st Weight (%)
Analyst:	EC	AG		
Tray color code:	Turquoise	Turquoise		
Date:	01-20-23	02-04-23 02-05-23		
A		20.02		
B		20.08		
C		22.95		
D		22.96		
E		23.08		
F		23.59		
G		23.54		
H		22.42		
I		21.30		
J		21.35		
K		19.72		
L		22.58		
M		21.07		
N		23.43		
O		23.43		
P		21.84		
Q		19.23		
R		17.86		
S		18.25		
T		20.06		
U		14.84		
V		13.67		
W		15.93		
X		15.67		
Y				
Z				
AA				
BB				

Average

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

Test type: P. promelas Chronic Reference Toxicant Test
 Test dates: January 10-17, 2023
 Associated test: PpKCICR # 98

1st Weight = Pans were dried for 24-hours at 60 ± 2°C, desiccated, and weighed following standard operating procedures.

2nd Weight = The same pans used for determining the 1st weight measurements were dried for an additional 24-hours at 60 ± 2°C, desiccated, and weighed following standard operating procedures.

Analyst: Tray color code: Date:	1st Weight	2nd Weight	Difference (mg)	Percent Difference from 1st Weight (%)
	EC	AG		
	Ruby	Ruby		
	01-20-23	02-05-23		
A	20.02	20.02	0.00	0.00
B	20.09	20.08	-0.01	-0.05
C	22.95	22.95	0.00	0.00
D	22.96	22.96	0.00	0.00
E	23.10	23.08	-0.02	-0.09
F	23.60	23.59	-0.01	-0.04
G	23.55	23.59	0.04	0.17
H	22.44	22.42	-0.02	-0.09
I	21.31	21.30	-0.01	-0.05
J	21.35	21.35	0.00	0.00
K	19.73	19.72	-0.01	-0.05
L	22.60	22.58	-0.02	-0.09
M	21.08	21.07	-0.01	-0.05
N	23.42	23.43	0.01	0.04
O	23.42	23.43	0.01	0.04
P	21.83	21.84	0.01	0.05
Q	19.25	19.23	-0.02	-0.10
R	17.87	17.86	-0.01	-0.06
S	18.25	18.25	0.00	0.00
T	20.07	20.06	-0.01	-0.05
U	14.83	14.84	0.01	0.07
V	13.67	13.67	0.00	0.00
W	15.93	15.93	0.00	0.00
X	15.67	15.67	0.00	0.00
Y	0.00	0.00		
Z	0.00	0.00		
AA	0.00	0.00		
BB	0.00	0.00		
	Average		0.00	-0.01

Species: Pimephales promelas

PpKCICR Test Number: 98

Daily Chemistry:

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

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		EC	EC	EC	U	U	U
Concentration	Parameter				011223		
CONTROL, MHSW	pH (S.U.)	8.01	7.70	7.83	7.88 ⁿ	7.87	7.71
	Dissolved oxygen (mg/L)	7.7	7.6	7.7	7.49 7.7	7.7	7.0
	Conductivity (µmhos/cm)	506		313		198	
	Alkalinity (mg CaCO ₃ /L)	63				63	
	Hardness (mg CaCO ₃ /L)	86				88	
	Temperature (°C)	24.9	24.8	24.8	24.4	24.7	24.5
300 mg KCl/L	pH (S.U.)	8.03	7.72	7.80	7.90 ⁰¹¹²²³	7.86	7.52
	Dissolved oxygen (mg/L)	7.7	7.6	7.6	7.46 7.6	7.0	6.2
	Conductivity (µmhos/cm)	868		892		837	
	Temperature (°C)	24.7	24.6	24.8	24.7	24.9	24.7
450 mg KCl/L	pH (S.U.)	8.03	7.71	7.88	7.45	7.90	7.50
	Dissolved oxygen (mg/L)	7.8	7.5	7.6	7.3	7.0	6.2
	Conductivity (µmhos/cm)	1120		1150		1110	
	Temperature (°C)	24.9	24.6	24.7	24.7	24.9	24.7
600 mg KCl/L	pH (S.U.)	8.02	7.72	7.88	7.41	7.92	7.53
	Dissolved oxygen (mg/L)	7.8	7.4	7.6	7.3	7.9	6.0
	Conductivity (µmhos/cm)	1390		1430		1360	
	Temperature (°C)	24.9	24.5	24.7	24.8	24.8	24.6
750 mg KCl/L	pH (S.U.)	8.01	7.72	7.92	7.42	7.91	7.52
	Dissolved oxygen (mg/L)	7.8	7.4	7.6	7.3	7.9	6.7
	Conductivity (µmhos/cm)	1640		1680		1610	
	Temperature (°C)	24.8	24.8	24.7	24.6	24.8	24.6
900 mg KCl/L	pH (S.U.)	8.02	7.77	7.92	7.42	7.93	7.46
	Dissolved oxygen (mg/L)	7.8	7.4	7.6	7.2	7.9	6.1
	Conductivity (µmhos/cm)	1920		1950		1870	
	Temperature (°C)	24.8	24.7	24.7	24.6	24.9	24.4
1050 mg KCl/L	pH (S.U.)	8.01	7.75	7.93	7.45	7.96	7.49
	Dissolved oxygen (mg/L)	7.8	7.6	7.6	7.2	7.8	7.0
	Conductivity (µmhos/cm)	2190		2270		2150	
	Temperature (°C)	24.9	24.7	24.7	24.7	24.9	24.7
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

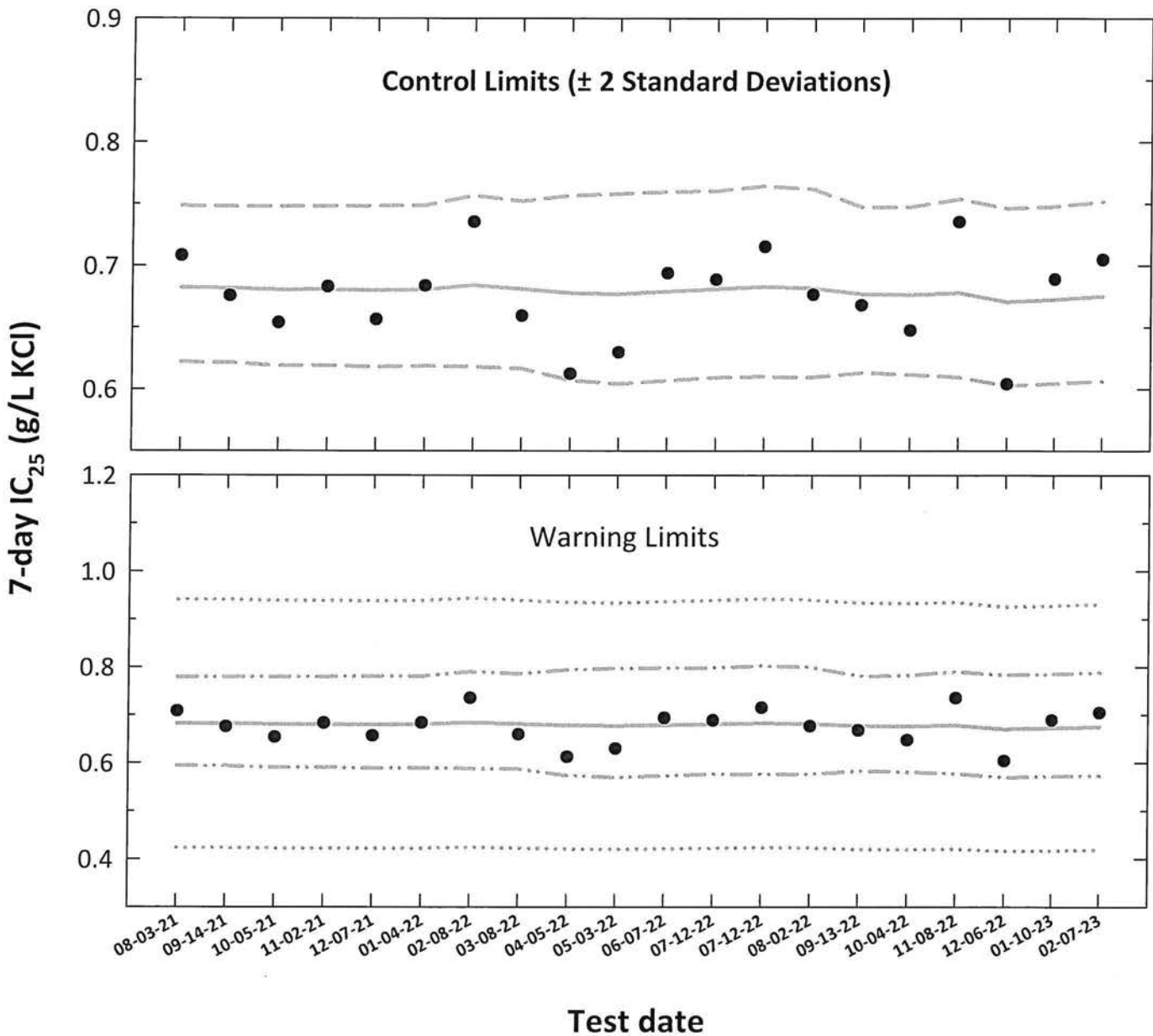
PpKICR Test Number: 98

Concentration		Parameter	Day							
			(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
			3		4		5		6	
Analyst		K	BSC	BSC	K	K	EC	EC	K	
CONTROL, MHSW	pH (S.U.)	8.07	7.84	7.97	7.62	8.00	7.73	7.92	7.65	
	Dissolved oxygen (mg/L)	7.6	7.8	7.8	7.3	7.0	7.3	7.0	7.0	
	Conductivity (µmhos/cm)	304		289		303		300		
	Alkalinity (mg CaCO ₃ /L)			62						
	Hardness (mg CaCO ₃ /L)			86						
	Temperature (°C)	24.6	24.7	24.8	24.5	24.7	24.3	24.8	24.8	
300 mg KCl/L	pH (S.U.)	8.02	7.90	8.08	7.45	7.97	7.68	7.97	7.46	
	Dissolved oxygen (mg/L)	7.5	7.8	8.0	7.3	7.0	7.3	7.9	6.5	
	Conductivity (µmhos/cm)	862		825		833		860		
	Temperature (°C)	24.7	24.9	24.8	24.4	24.7	24.6	24.8	24.4	
450 mg KCl/L	pH (S.U.)	8.03	7.79	8.10	7.45	7.95	7.64	7.98	7.45	
	Dissolved oxygen (mg/L)	7.5	7.8	8.0	7.0	7.0	7.3	7.9	6.6	
	Conductivity (µmhos/cm)	1090		1060		1060		1090		
	Temperature (°C)	24.7	24.9	24.9	24.6	24.7	24.5	24.8	24.4	
600 mg KCl/L	pH (S.U.)	8.05	7.81	8.10	7.43	7.95	7.65	7.97	7.43	
	Dissolved oxygen (mg/L)	7.6	7.6	8.0	7.0	7.9	7.4	7.9	6.4	
	Conductivity (µmhos/cm)	1350		1330		1330		1350		
	Temperature (°C)	24.7	25.0	24.8	24.6	24.8	24.6	24.7	24.6	
750 mg KCl/L	pH (S.U.)	8.02	7.77	8.11	7.43	7.94	7.66	7.96	7.44	
	Dissolved oxygen (mg/L)	7.6	7.7	8.0	7.0	7.9	7.5	8.0	6.4	
	Conductivity (µmhos/cm)	1620		1570		1580		1600		
	Temperature (°C)	24.8	25.0	24.9	24.6	24.8	24.6	24.7	24.6	
900 mg KCl/L	pH (S.U.)	8.02	7.77	8.11	7.42	7.94	7.74	7.96	7.50	
	Dissolved oxygen (mg/L)	7.6	7.7	8.0	7.0	8.0	7.6	8.0	6.4	
	Conductivity (µmhos/cm)	1910		1850		1800		1850		
	Temperature (°C)	24.8	24.7	24.9	24.6	24.8	24.6	24.8	24.3	
1050 mg KCl/L	pH (S.U.)	8.03	7.75	8.12	7.49	7.98	7.76	7.96	7.48	
	Dissolved oxygen (mg/L)	7.7	7.7	8.0	7.4	8.0	7.6	8.0	6.5	
	Conductivity (µmhos/cm)	2170		2100		2090		2140		
	Temperature (°C)	25.0	24.7	24.9	24.7	24.8	24.3	24.7	24.6	
		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 \pm 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic IC₂₅ \pm 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic IC₂₅ \pm S_{A,75} converted to anti-logarithmic values, S_{A,75} = 75th percentile of CVs reported nationally by USEPA)

Pimephales promelas

Chronic Reference Toxicant Control Chart

Source: In-house Culture

Test number	Test date	7-day IC ₂₅ ToxCal Determination (g/L KCl)	Log ₁₀ Conversion		Anti-logarithmic Values (g/L KCl)							
			7-day IC ₂₅	CT	S	CT	Control Limits		Laboratory Calculated CV		75th Percentile CV	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV		CT - S _{A,75}
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	07-12-22	0.6887	-0.1620	-0.1669	0.0240	0.6810	0.6096	0.7607	0.5761	0.7980	0.4222	0.9397
13	07-12-22	0.7153	-0.1455	-0.1655	0.0245	0.6831	0.6104	0.7646	0.5767	0.8023	0.4235	0.9427
14	08-02-22	0.6766	-0.1697	-0.1665	0.0242	0.6816	0.6097	0.7620	0.5762	0.7995	0.4226	0.9406
15	09-13-22	0.6682	-0.1751	-0.1693	0.0215	0.6772	0.6135	0.7475	0.5831	0.7810	0.4199	0.9345
16	10-04-22	0.6477	-0.1886	-0.1698	0.0218	0.6764	0.6118	0.7478	0.5809	0.7820	0.4194	0.9334
17	11-08-22	0.7354	-0.1335	-0.1687	0.0230	0.6781	0.6098	0.7540	0.5774	0.7900	0.4204	0.9357
18	12-06-22	0.6041	-0.2189	-0.1735	0.0232	0.6707	0.6029	0.7462	0.5695	0.7833	0.4158	0.9256
19	01-10-23	0.6890	-0.1618	-0.1722	0.0231	0.6726	0.6047	0.7481	0.5717	0.7848	0.4170	0.9282
20	02-07-23	0.7050	-0.1518	-0.1706	0.0233	0.6752	0.6064	0.7518	0.5733	0.7886	0.4186	0.9317

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.

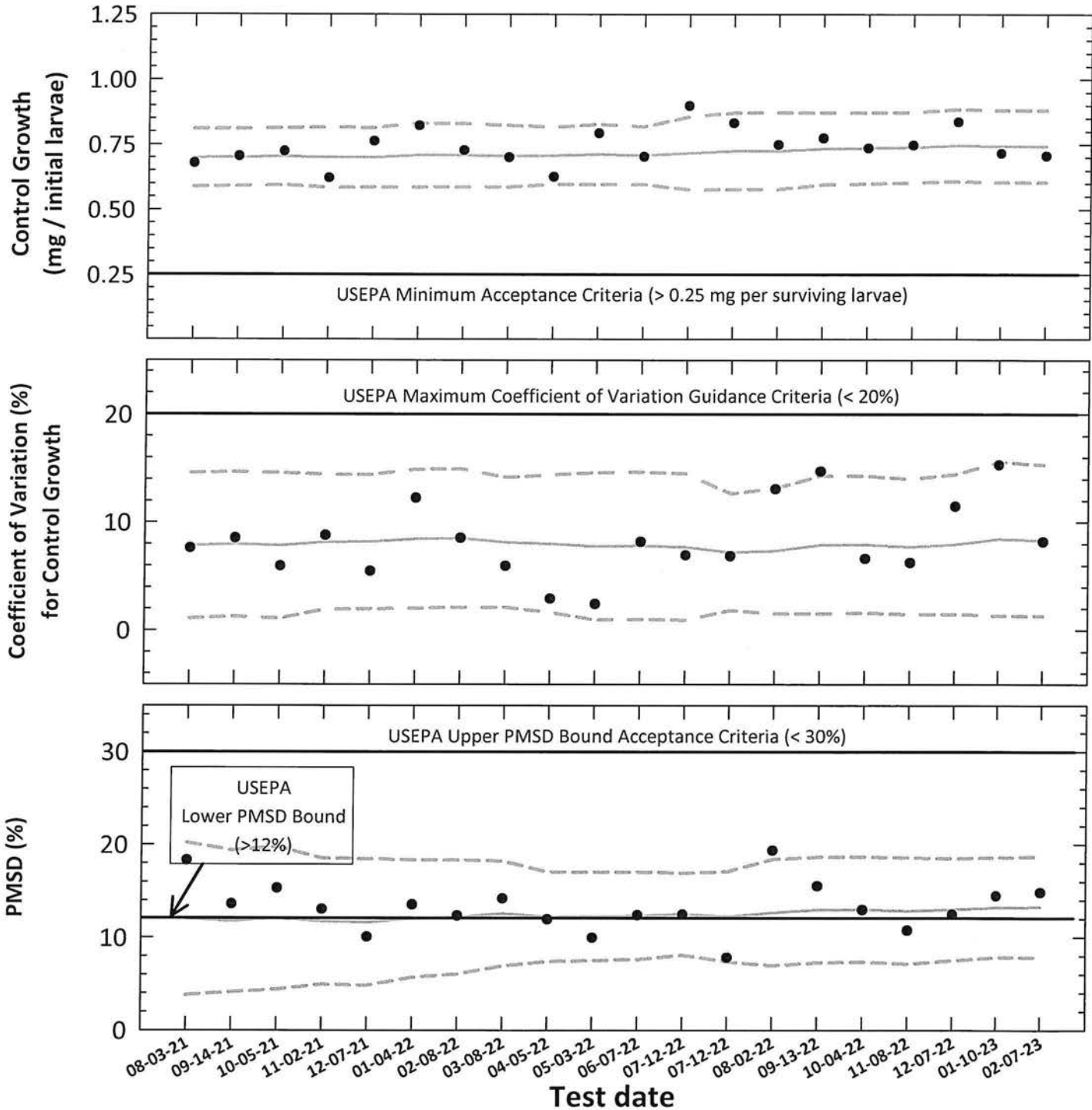
Warning Limits = Mean logarithmic IC₂₅ ± 2CV or S_{A,75} converted to anti-logarithmic values.

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)

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

Test number	Test date	ToxCal Determination				Control Growth		Control Growth CV		Test PMSD				
		Control Survival (%)	Control Growth		PMSD (%)	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT - 2S	CT	95% Confidence Interval CT + 2S			
			Mean (mg/initial larvae)	CV (%)								MSD	Test	
1	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	1.1	12.0	3.8	20.2
2	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	1.3	11.7	4.1	19.4
3	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	12.1	4.4	19.7
4	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	11.7	4.9	18.5
5	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	11.6	4.8	18.5
6	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	12.0	5.7	18.3
7	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	12.2	6.0	18.3
8	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	12.6	6.9	18.2
9	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	12.2	7.4	17.0
10	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	12.3	7.5	17.0
11	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	12.3	7.6	17.0
12	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	12.5	8.1	16.9
13	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.2	7.4	17.1
14	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	1.5	12.7	6.9	18.4
15	09-13-22	100	0.774	14.7	0.1203	15.5	0.734	0.596	0.872	7.9	1.5	13.0	7.3	18.7
16	10-04-22	100	0.736	6.6	0.0955	13.0	0.736	0.600	0.873	7.9	1.6	13.0	7.4	18.7
17	11-08-22	100	0.747	6.3	0.0804	10.8	0.739	0.604	0.874	7.7	1.5	12.9	7.1	18.6
18	12-07-22	100	0.837	11.5	0.1043	12.5	0.747	0.608	0.886	8.0	1.5	13.0	7.5	18.5
19	01-10-23	100	0.716	15.3	0.1037	14.5	0.744	0.605	0.882	8.5	1.3	13.2	7.9	18.6
20	02-07-23	100	0.707	8.2	0.1049	14.8	0.743	0.604	0.882	8.3	1.3	13.3	7.9	18.7

Note: Control Survival = USEPA minimum test acceptability criteria \geq 80% survival.
Control Mean Growth = USEPA minimum test acceptability criteria \geq 0.25 mg/surviving larvae.
CV = Coefficient of variation for control growth.
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: 99

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>2166</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>01-31-23</u>	Artemia CHM number:	CHM1222
Hatch dates and times:	<u>02-06-23 1300 TO 02-07-23 0600</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>7.72</u> S.U. Temperature = <u>24.9</u> °C	Date / Time in oven:	<u>02-14-23 1105</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>02-15-23 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	02-07-23	0505	J	1117	J	0715	J	02-01-23 B
1	02-08-23	0500	J	1100	J	0700	J	↓
2	02-09-23	0500	J	1100	J	0700	J	02-01-23 D
3	02-10-23	0500	J	1200	J	0700	J	↓
4	02-11-23	0600	J	1200	J	0800	J	02-09-23 A
5	02-12-23	0500	J	1100	H	0700	J	↓
6	02-13-23	0500	J	1100	H	0700	H	↓
7	02-14-23					0615	J	

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>774.7</u>
Average weight per initial larvae:	<u>0.707</u>		NOEC (mg/L KCl)	<u>450</u>
Average weight per surviving larvae:	<u>0.707</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>705.0</u>

Species: Pimephales promelas

PpKCICR Test Number: 99

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>ALB</u> Date: <u>01-31-23</u>	15.60	15.30	15.12	14.90	13.21	13.72	14.40	14.89	14.41	12.96	14.16	12.61
*B = Pan + Larvae weight (mg) Analyst: <u>ALB</u> Date: <u>02-16-23</u>	22.49	22.46	22.92	21.31	21.26	21.75	22.34	22.71	23.64	19.88	21.57	19.99
C = Larvae weight (mg) = B - A Analyst: <u>ALB</u>	6.89	7.16	7.80	6.41	7.99	8.03	7.94	7.82	9.23	6.92	7.41	7.38
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>ALB</u>	0.689	0.716	0.780	0.641	0.799	0.803	0.794	0.782	0.923	0.692	0.741	0.738
Average weight per initial number of larvae (mg)	0.707				0.795				0.774			
Percent reduction from control (%)					-12.57.				-9.57.			

*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: 99

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L						
	M	N	O	P	Q	R	S	T	U	V	W	X			
0	10	10	10	10	10	10	10	10	10	10	10	10			
1	10	10	10	10	9 ^{id}	10	9 ^{id}	9 ^{id}	6 ^{4d}	5 ^{5d}	6 ^{4d}	5 ^{5d}			
2	10	10	10	10	9	9 ^{id}	9	8 ^{id}	5 ^{id}	5	5 ^{id}	4 ^{id}			
3	10	10	10	10	9	9	9	8	4 ^{id}	5	4 ^{id}	4			
4	9 ^{unusually small}	10	10	10	7 ^{2d}	8 ^{id}	7 ^{2d}	7 ^{id}	3 ^{id}	3 ^{2d}	3 ^{id}	4			
5	9	10	10	10	7 ^{id}	8	6 ^{id}	7	3	3	2 ^{id}	3 ^{id}			
6	9	9 ^{id}	9 ^{id}	9 ^{id}	6	6 ^{2d}	6	7	3	2 ^{id}	2	2 ^{id}			
7	9	9	9	9	6	6	6	7	2 ^{id}	2	2	2			
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>AL</u> Date: <u>01-31-23</u>															
*B = Pan + Larvae weight (mg) Analyst: <u>AL</u> Date: <u>02-16-23</u>															
C = Larvae weight (mg) = B - A Analyst: <u>AL</u>															
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>AL</u>															
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		0.726		-2.77		0.501		29.17		0.198		72.07	

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

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

Comments:

Species: *Pimephales promelas*

PpKICR Test Number: 99

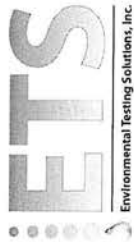
Survival and Growth Data

Day	1050 mg KCl/L				
	Y	Z	AA	BB	
0	10	10	10	10	
1	3 ^{7d}	4 ^{6d}	2 ^{8d}	3 ^{7d}	
2	2 ^{2d} 3 ^{2d} 0 ^{sk}	2 ^{2d}	2	1 ^{2d}	
3	2	2	1 ^{1d}	1	
4	1 ^{1d}	0 ^{2d}	1	1	
5	1	0	0 ^{1d}	1	
6	0 ^{1d}	0	0	1	
7	0	0	0	1	
*A = Pan weight (mg) Tray color code: <u>light pink</u> Analyst: <u>AG</u> Date: <u>01-31-23</u>		14.62	15.55	14.60	14.71
*B = Pan + Larvae weight (mg) Analyst: <u>AG</u> Date: <u>02-16-23</u>				15.46	
C = Larvae weight (mg) = B - A Analyst: <u>J</u>				0.75	
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>J</u>		0	0	0	0.075
Average weight per initial number of larvae (mg)	Percent reduction from control (%)	0.014		97.37	

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

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

Comments:



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

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

Test number: 99
Test dates: February 07-14, 2023

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pin weight (mg)	B = Pin + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight / Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight / Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	15.60	22.49	6.89	0.689			0.689				
	B	10	10	15.30	22.46	7.16	0.716	0.707	8.2	0.716	100.0	0.707	8.2	Not applicable
	C	10	10	15.12	22.92	7.80	0.780			0.780				
	D	10	10	14.90	21.31	6.41	0.641			0.641				
300	E	10	10	13.21	21.20	7.99	0.799	0.795	1.1	0.799	100.0	0.795	1.1	-12.5
	F	10	10	13.72	21.75	8.03	0.803			0.803				
	G	10	10	14.40	22.34	7.94	0.794			0.794				
	H	10	10	14.89	22.71	7.82	0.782			0.782				
450	I	10	10	14.41	23.64	9.23	0.923	0.774	13.2	0.923	100.0	0.774	13.2	-9.5
	J	10	10	12.96	19.88	6.92	0.692			0.692				
	K	10	10	14.16	21.57	7.41	0.741			0.741				
	L	10	10	12.61	19.99	7.38	0.738			0.738				
600	M	10	9	14.29	21.88	7.59	0.843	0.806	3.4	0.759	90.0	0.726	3.4	-2.7
	N	10	9	13.17	20.33	7.16	0.796			0.716				
	O	10	9	13.42	20.69	7.27	0.808			0.727				
	P	10	9	15.38	22.39	7.01	0.779			0.701				
750	Q	10	6	14.48	19.21	4.73	0.788	0.802	3.3	0.473	62.5	0.501	7.0	29.1
	R	10	6	15.90	20.67	4.77	0.795			0.477				
	S	10	6	12.65	17.70	5.05	0.842			0.505				
	T	10	7	13.51	19.00	5.49	0.784			0.549				
900	U	10	2	14.05	15.94	1.89	0.945	0.990	8.5	0.189	20.0	0.198	8.5	72.0
	V	10	2	14.42	16.64	2.22	1.110			0.222				
	W	10	2	13.95	15.79	1.84	0.920			0.184				
	X	10	2	14.70	16.67	1.97	0.985			0.197				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.750	0.0	0.000	2.5	0.019	0.0	97.3
	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	1	14.71	15.46	0.75	0.750			0.075				

Dummett's MSD value: 0.1049
PMSD: 14.8
MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

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





Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 2/7/2023	Test ID: PpKClCR	Sample ID: REF-Ref Toxicant
End Date: 2/14/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas

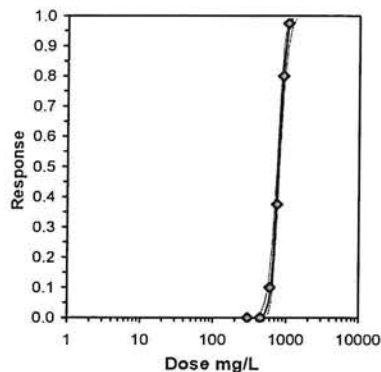
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	0.9000	0.9000	0.9000	0.9000
750	0.6000	0.6000	0.6000	0.7000
900	0.2000	0.2000	0.2000	0.2000
1050	0.0000	0.0000	0.0000	0.1000

Conc-mg/L	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%	N				
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*600	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	4	10.00	10.00	4	40
*750	0.6250	0.6250	0.9123	0.8861	0.9912	5.759	4	10.00	10.00	15	40
*900	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	4	10.00	10.00	32	40
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

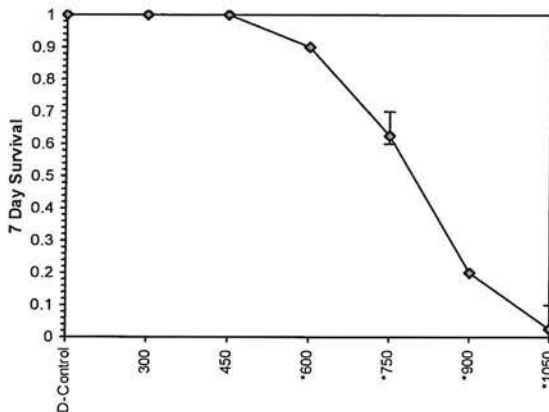
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.61099	0.896	2.43204	8.14858
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	450	600	519.615	

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	13.1425	1.61501	9.97712	16.308	0	1.20348	9.48773	0.87753	2.88912	0.07609	3
Intercept	-32.97	4.68262	-42.148	-23.792							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	515.36	445.956	564.918
EC05	3.355	580.717	520.386	623.801
EC10	3.718	618.882	564.502	658.26
EC15	3.964	646.039	595.992	683.001
EC20	4.158	668.47	621.942	703.693
EC25	4.326	688.333	644.783	722.304
EC40	4.747	741.042	703.989	773.753
EC50	5.000	774.675	739.992	808.852
EC60	5.253	809.835	775.654	847.923
EC75	5.674	871.848	833.773	922.596
EC80	5.842	897.755	856.619	955.59
EC85	6.036	928.925	883.302	996.373
EC90	6.282	969.687	917.187	1051.17
EC95	6.645	1033.41	968.545	1139.46
EC99	7.326	1164.47	1070.24	1328.72



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

Larval Fish Growth and Survival Test-7 Day Growth

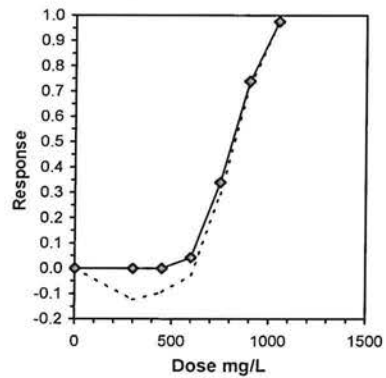
Start Date: 2/7/2023	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 2/14/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas

Conc-mg/L	1	2	3	4
D-Control	0.6890	0.7160	0.7800	0.6410
300	0.7990	0.8030	0.7940	0.7820
450	0.9230	0.6920	0.7410	0.7380
600	0.7590	0.7160	0.7270	0.7010
750	0.4730	0.4780	0.5050	0.5490
900	0.1890	0.2220	0.1840	0.1970
1050	0.0000	0.0000	0.0000	0.0750

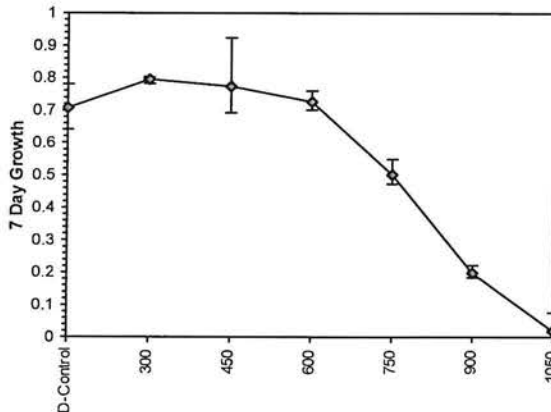
Conc-mg/L	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
D-Control	0.7065	1.0000	0.7065	0.6410	0.7800	8.208	4			0.7582	1.0000
300	0.7945	1.1246	0.7945	0.7820	0.8030	1.147	4	26.00	11.00	0.7582	1.0000
450	0.7735	1.0948	0.7735	0.6920	0.9230	13.207	4	22.00	11.00	0.7582	1.0000
600	0.7258	1.0272	0.7258	0.7010	0.7590	3.389	4			0.7258	0.9572
750	0.5013	0.7095	0.5013	0.4730	0.5490	6.942	4			0.5013	0.6611
900	0.1980	0.2803	0.1980	0.1840	0.2220	8.521	4			0.1980	0.2612
1050	0.0188	0.0265	0.0188	0.0000	0.0750	200.000	4			0.0188	0.0247

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.8865	0.805	1.32581	2.49725
Bartlett's Test indicates unequal variances (p = 9.98E-03)	9.21519	9.21035		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	450	>450		

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	603.67	54.23	310.78	625.89	-1.7657
IC10	629.00	10.08	591.42	650.39	-0.1013
IC15	654.33	9.59	618.98	677.24	0.0417
IC20	679.65	9.51	646.49	706.06	0.2103
IC25	704.98	9.85	672.36	735.91	0.3628
IC40	772.93	7.31	747.64	790.93	0.0135
IC50	810.43	5.66	791.00	823.04	-0.0140



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

Statistical Analyses

Used for PMSD calculation only.

Larval Fish Growth and Survival Test-7 Day Growth

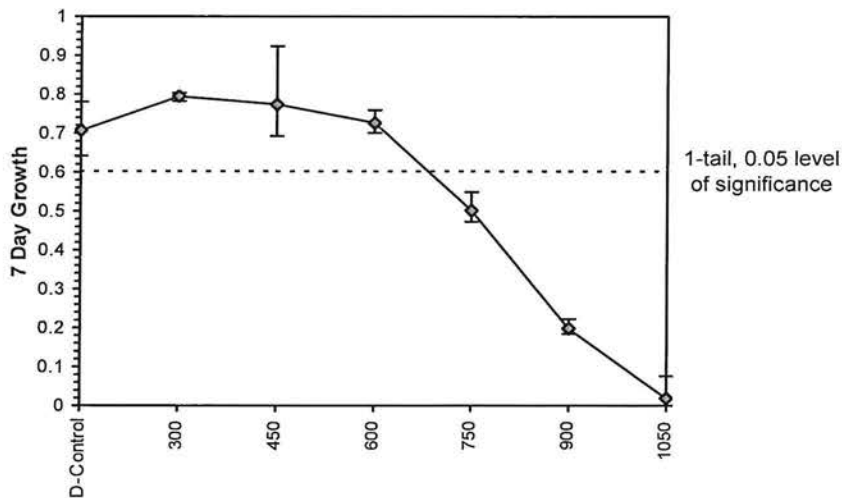
Start Date: 2/7/2023	Test ID: PpKCICR	Sample ID: REF-Ref Toxicant
End Date: 2/14/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas
Comments:		

Conc-mg/L	1	2	3	4
D-Control	0.6890	0.7160	0.7800	0.6410
300	0.7990	0.8030	0.7940	0.7820
450	0.9230	0.6920	0.7410	0.7380
600	0.7590	0.7160	0.7270	0.7010
750	0.4730	0.4780	0.5050	0.5490
900	0.1890	0.2220	0.1840	0.1970
1050	0.0000	0.0000	0.0000	0.0750

Conc-mg/L	Transform: Untransformed							1-Tailed		
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.7065	1.0000	0.7065	0.6410	0.7800	8.208	4			
300	0.7945	1.1246	0.7945	0.7820	0.8030	1.147	4	-1.829	2.180	0.1049
450	0.7735	1.0948	0.7735	0.6920	0.9230	13.207	4	-1.393	2.180	0.1049
600	0.7258	1.0272	0.7258	0.7010	0.7590	3.389	4			
750	0.5013	0.7095	0.5013	0.4730	0.5490	6.942	4			
900	0.1980	0.2803	0.1980	0.1840	0.2220	8.521	4			
1050	0.0188	0.0265	0.0188	0.0000	0.0750	200.000	4			

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.8865	0.805	1.32581	2.49725						
Bartlett's Test indicates unequal variances ($p = 9.98E-03$)	9.21519	9.21035								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	>450			0.10486	0.14842	0.00845	0.00463	0.21597	2, 9
Treatments vs D-Control										

Dose-Response Plot



Species: Pimephales promelas

PpKCICR Test Number: 99

Daily Chemistry:

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

Analyst		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
		N	N	N	N	N	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.38	7.20	7.41	7.31	7.38	7.24
	Dissolved oxygen (mg/L)	7.0	7.4	7.0	7.4	8.0	7.6
	Conductivity (µmhos/cm)	293		320		317	
	Alkalinity (mg CaCO ₃ /L)	60				61	
	Hardness (mg CaCO ₃ /L)	86				94	
	Temperature (°C)	24.8	24.5	24.8	24.8	24.7	25.0
300 mg KCl/L	pH (S.U.)	7.68	7.37	7.64	7.29	7.54	7.62
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.5	7.9	7.7
	Conductivity (µmhos/cm)	845		867		836	
	Temperature (°C)	24.9	24.7	24.7	24.6	24.7	24.8
450 mg KCl/L	pH (S.U.)	7.64	7.35	7.64	7.34	7.53	7.61
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.6	7.9	7.8
	Conductivity (µmhos/cm)	1080		1110		1110	
	Temperature (°C)	24.9	24.7	24.7	24.6	24.7	24.8
600 mg KCl/L	pH (S.U.)	7.65	7.46	7.67	7.35	7.50	7.61
	Dissolved oxygen (mg/L)	7.7	7.8	7.9	7.6	7.8	7.8
	Conductivity (µmhos/cm)	1340		1400		1340	
	Temperature (°C)	24.9	24.6	24.7	24.7	24.8	25.1
750 mg KCl/L	pH (S.U.)	7.72	7.41	7.69	7.38	7.59	7.62
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.6	7.9	7.8
	Conductivity (µmhos/cm)	1600		1650		1620	
	Temperature (°C)	24.7	24.6	24.7	24.7	24.8	25.1
900 mg KCl/L	pH (S.U.)	7.74	7.59	7.71	7.39	7.62	7.64
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.5	7.9	7.7
	Conductivity (µmhos/cm)	1850		1940		1870	
	Temperature (°C)	24.8	24.5	24.7	24.7	24.7	24.9
1050 mg KCl/L	pH (S.U.)	7.76	7.54	7.72	7.45	7.65	7.73
	Dissolved oxygen (mg/L)	7.9	7.8	8.0	7.8	7.9	7.9
	Conductivity (µmhos/cm)	2140		2200		2160	
	Temperature (°C)	24.8	24.7	24.7	24.4	24.7	24.9
		Initial	Final	Initial	Final	Initial	Final

Species: Pimephales promelas

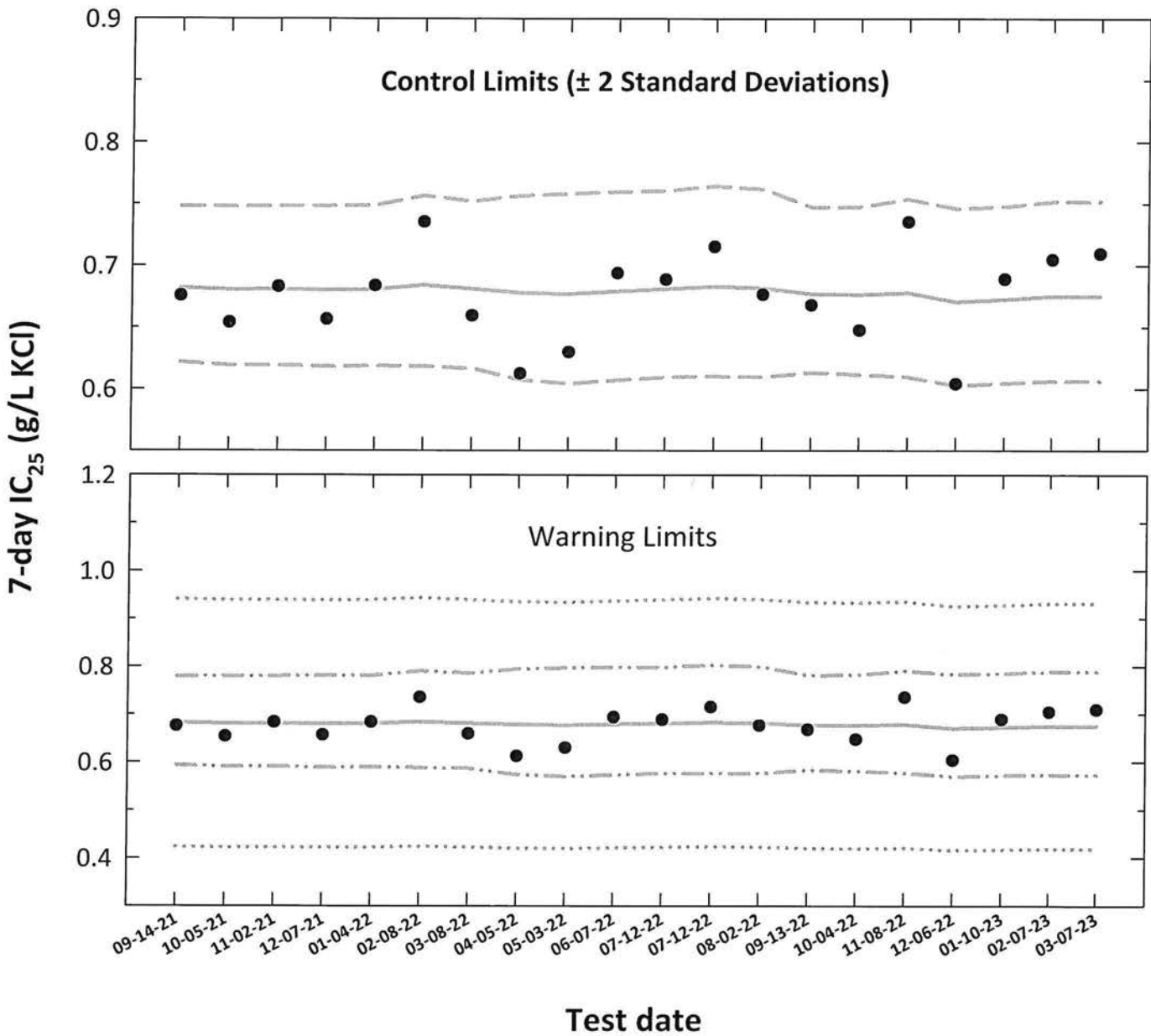
PpKCICR Test Number: 99

Concentration		Parameter	Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
			3		4		5		6	
			Analyst							
CONTROL, MHSW	pH (S.U.)	8.08	7.87	8.10	7.69	8.04	7.69	8.01	7.55	
	Dissolved oxygen (mg/L)	7.8	7.5	7.9	7.0	7.6	7.7	7.7	6.4	
	Conductivity (µmhos/cm)	311		288		319		310		
	Alkalinity (mg CaCO ₃ /L)			61						
	Hardness (mg CaCO ₃ /L)			82						
	Temperature (°C)	24.7	24.6	24.7	24.7	24.8	24.6	24.7	24.8	
300 mg KCl/L	pH (S.U.)	7.99	7.79	8.09	7.53	8.07	7.64	8.10	7.41	
	Dissolved oxygen (mg/L)	8.0	7.6	8.0	7.0	7.6	7.2	8.0	6.3	
	Conductivity (µmhos/cm)	817		804		867		832		
	Temperature (°C)	24.6	24.4	24.8	24.5	24.7	24.5	24.8	24.7	
450 mg KCl/L	pH (S.U.)	7.90	7.79	8.11	7.58	8.09	7.65	8.07	7.41	
	Dissolved oxygen (mg/L)	8.0	7.6	8.0	6.8	7.7	7.2	7.9	6.2	
	Conductivity (µmhos/cm)	1070		1050		1130		1070		
	Temperature (°C)	24.7	24.4	24.8	24.7	24.7	24.5	24.7	24.7	
600 mg KCl/L	pH (S.U.)	7.94	7.85 7.85	8.11	7.49	8.08	7.70	8.07	7.40	
	Dissolved oxygen (mg/L)	8.0	7.7	8.0	6.7	7.7	7.2	7.9	6.0	
	Conductivity (µmhos/cm)	1330		1300		1410		1340		
	Temperature (°C)	24.7	24.8	24.9	24.7	24.7	24.7	24.7	24.7	
750 mg KCl/L	pH (S.U.)	7.95	7.80	8.11	7.47	8.09	7.70	8.06	7.30	
	Dissolved oxygen (mg/L)	8.0	7.8	8.0	6.1	7.7	7.5	7.9	6.1	
	Conductivity (µmhos/cm)	1500		1540		1630		1580		
	Temperature (°C)	24.7	24.8	24.9	24.6	24.7	24.7	24.8	24.9	
900 mg KCl/L	pH (S.U.)	7.95	7.86	8.11	7.57	8.10	7.77	8.06	7.42	
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	6.8	7.7	7.6	8.0	5.8	
	Conductivity (µmhos/cm)	1850		1790		1980		1840		
	Temperature (°C)	24.8	24.7	24.9	24.6	24.8	24.8	24.8	24.9	
1050 mg KCl/L	pH (S.U.)	7.97	7.85	8.09	7.57	8.09	7.46	8.06	7.44	
	Dissolved oxygen (mg/L)	8.1	7.9	8.0	6.9	7.7	7.4	8.0	6.0	
	Conductivity (µmhos/cm)	2090		2030		2170		2090		
	Temperature (°C)	24.8	24.7	24.8	24.6	24.8	24.6	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 IC₂₅** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC₂₅ converted to anti-logarithmic values)
- - - **Control Limits** (mean logarithmic IC₂₅ ± 2 standard deviations converted to anti-logarithmic values)
- . . . **Laboratory Warning Limits** (mean logarithmic IC₂₅ ± 2 coefficient of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic IC₂₅ ± S_{A,75} converted to anti-logarithmic values, S_{A,75} = 75th percentile of CVs reported nationally by USEPA)

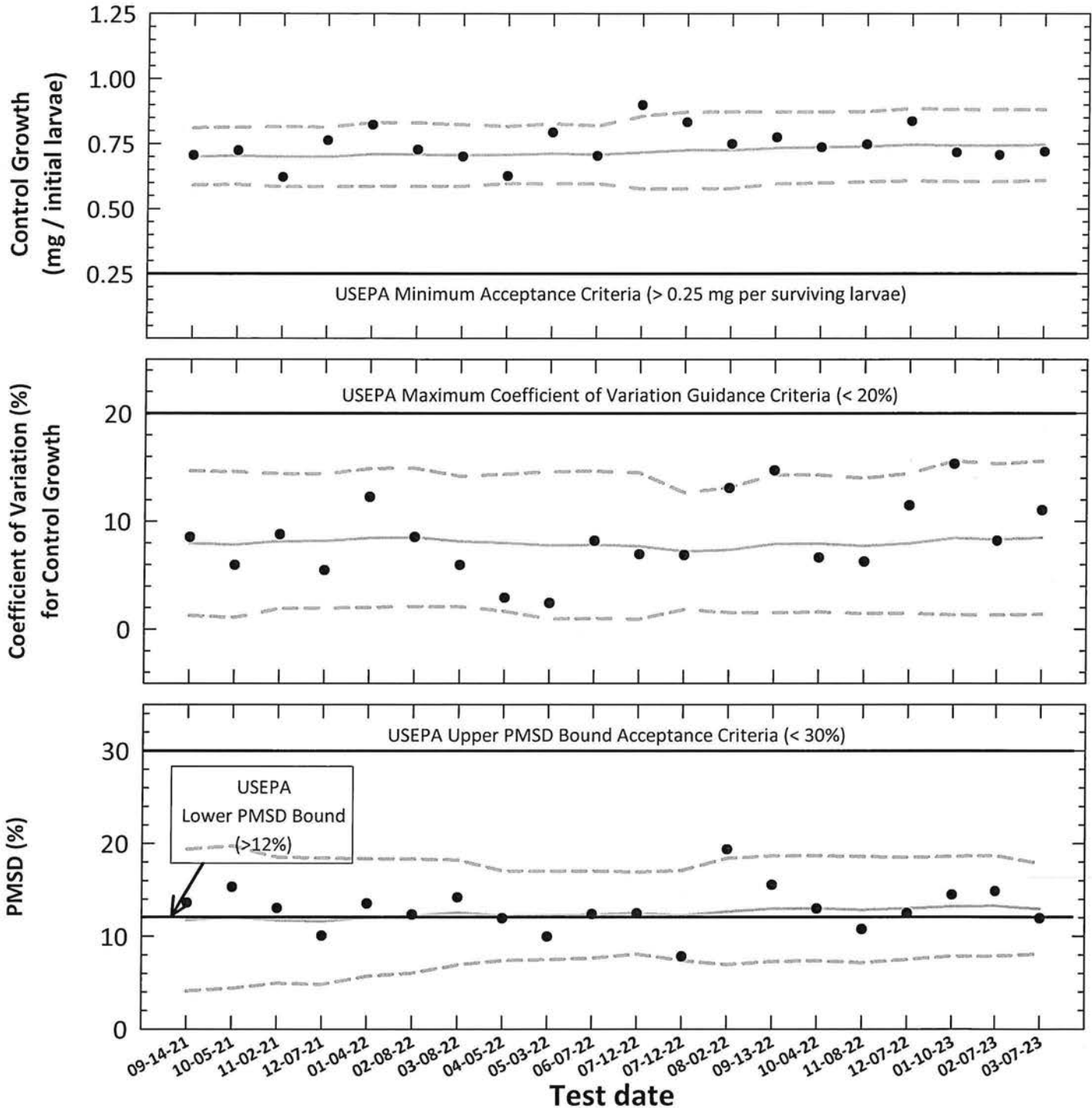
Pimephales promelas
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Source: In-house Culture

Test number	Test date	ToxCal Determination					Control Growth			Control Growth CV			Test PMSD	
		Control Survival (%)	Control Growth		MSD	PMSD (%)	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval
			Mean (mg/initial larvae)	CV (%)				CT -2S	CT +2S		CT -2S	CT +2S		
1	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	1.3	11.7	4.1	19.4
2	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	12.1	4.4	19.7
3	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	11.7	4.9	18.5
4	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	11.6	4.8	18.5
5	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	12.0	5.7	18.3
6	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	12.2	6.0	18.3
7	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	12.6	6.9	18.2
8	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	12.2	7.4	17.0
9	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	12.3	7.5	17.0
10	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	12.3	7.6	17.0
11	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	12.5	8.1	16.9
12	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.2	7.4	17.1
13	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	1.5	12.7	6.9	18.4
14	09-13-22	100	0.774	14.7	0.1203	15.5	0.734	0.596	0.872	7.9	1.5	13.0	7.3	18.7
15	10-04-22	100	0.736	6.6	0.0955	13.0	0.736	0.600	0.873	7.9	1.6	13.0	7.4	18.7
16	11-08-22	100	0.747	6.3	0.0804	10.8	0.739	0.604	0.874	7.7	1.5	12.9	7.1	18.6
17	12-07-22	100	0.837	11.5	0.1043	12.5	0.747	0.608	0.886	8.0	1.5	13.0	7.5	18.5
18	01-10-23	100	0.716	15.3	0.1037	14.5	0.744	0.605	0.882	8.5	1.3	13.2	7.9	18.6
19	02-07-23	100	0.707	8.2	0.1049	14.8	0.743	0.604	0.882	8.3	1.3	13.3	7.9	18.7
20	03-07-23	100	0.719	11.0	0.0858	11.9	0.745	0.609	0.881	8.5	1.4	13.0	8.1	17.8

Note:
Control Survival = USEPA minimum test acceptability criteria \geq 80% survival.
Control Mean Growth = USEPA minimum test acceptability criteria \geq 0.25 mg/surviving larvae.
CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%
PMSD = Minimum significant difference.
PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 12%.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.
CT = Central tendency of the growth, CV or PMSD values.
S = Standard deviation of the growth, CV or PMSD values.



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)

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	07-12-22	0.6887	-0.1620	-0.1669	0.0240	0.6810	0.6096	0.7607	0.5761	0.7980	0.4222	0.9397
12	07-12-22	0.7153	-0.1455	-0.1655	0.0245	0.6831	0.6104	0.7646	0.5767	0.8023	0.4235	0.9427
13	08-02-22	0.6766	-0.1697	-0.1665	0.0242	0.6816	0.6097	0.7620	0.5762	0.7995	0.4226	0.9406
14	09-13-22	0.6682	-0.1751	-0.1693	0.0215	0.6772	0.6135	0.7475	0.5831	0.7810	0.4199	0.9345
15	10-04-22	0.6477	-0.1886	-0.1698	0.0218	0.6764	0.6118	0.7478	0.5809	0.7820	0.4194	0.9334
16	11-08-22	0.7354	-0.1335	-0.1687	0.0230	0.6781	0.6098	0.7540	0.5774	0.7900	0.4204	0.9357
17	12-06-22	0.6041	-0.2189	-0.1735	0.0232	0.6707	0.6029	0.7462	0.5695	0.7833	0.4158	0.9256
18	01-10-23	0.6890	-0.1618	-0.1722	0.0231	0.6726	0.6047	0.7481	0.5717	0.7848	0.4170	0.9282
19	02-07-23	0.7050	-0.1518	-0.1706	0.0233	0.6752	0.6064	0.7518	0.5733	0.7886	0.4186	0.9317
20	03-07-23	0.7096	-0.1490	-0.1706	0.0234	0.6752	0.6063	0.7520	0.5732	0.7889	0.4186	0.9318

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.

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

PpKCICR Test Number: 100

Dilution preparation information:							Comments:
KCl Stock INSS number:		INSS <u>7166</u>					
Stock preparation:		50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.					
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		Test information:	
Organism source:	In-house culture	Randomizing template:	<u>yellow</u>
Age:	< 24-hours old	Incubator number and shelf location:	<u>7B</u>
Spawn date:	<u>03-01-23</u>	Artemia CHM number:	CHM1222
Hatch dates and times:	<u>03-06-23 1425 TO 03-07-23 0500</u>	Drying information for weight determination:	
Transfer vessel information:	pH = <u>8.15</u> S.U. Temperature = <u>24.4</u> °C	Date / Time in oven:	<u>03-14-23 0720</u>
Average transfer volume:	< 0.25 mL	*Initial oven temperature:	<u>60 °C</u>
		Date / Time out of oven:	<u>03-15-23 0720</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	03-07-23	0504	JL	1110	JL	0714	JL	02-28-23 A
1	03-08-23	0500	JL	1100	JL	0710	JL	↓
2	03-09-23	0500	JL	1100	JL	0710	JL	02-28-23 D
3	03-10-23	0500	JL	1100	JL	0700	JL	↓
4	03-11-23	0600	JL	1200	JL	0800	JL	03-06-23 A
5	03-12-23	0600	JL	1100	JL	0800	JL	↓
6	03-13-23	0500	JL	1100	JL	0700	JL	↓
7	03-14-23					0645	JL	

Chemical analyses:

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

Control information:		Acceptance criteria	Summary of test endpoints:	
% Mortality:	<u>07.</u>	≤ 20%	7-day LC ₅₀ (mg/L KCl)	<u>788.1</u>
Average weight per initial larvae:	<u>0.719</u>		NOEC (mg/L KCl)	<u>600</u>
Average weight per surviving larvae:	<u>0.719</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>709.6</u>

Species: Pimephales promelas

PpKCICR Test Number: 100

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>02-22-23 BL Navy Grey</u> Analyst: <u>BL</u> Date: <u>02-22-23</u>	14.37	14.82	14.84	13.44	14.06	13.50	15.90	14.92	13.18	14.38	13.94	13.99
*B = Pan + Larvae weight (mg) Analyst: <u>BL</u> Date: <u>03-16-23</u>	20.77	23.08	21.69	20.68	22.70	21.83	23.58	22.50	20.17	21.63	21.20	21.65
C = Larvae weight (mg) = B - A Analyst: <u>JL</u>	6.40	8.26	6.85	7.24	8.64	8.33	7.78	7.58	6.99	7.25	7.26	7.66
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>JL</u>	0.640	0.826	0.685	0.724	0.864	0.833	0.778	0.758	0.699	0.725	0.726	0.766
Average weight per initial number of larvae (mg)	0.719				0.808		-12.57		0.729		-1.47	
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: 100

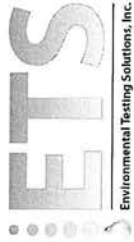
Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L						
	M	N	O	P	Q	R	S	T	U	V	W	X			
0	10	10	10	10	10	10	10	10	10	10	10	10			
1	10	10	10	10	9 ^{1d}	9 ^{1d}	10	10	5 ^{sd}	5 ^{sd}	5 ^{sd}	6 ^{1d}			
2	10	10	10	10	9	9	9 ^{1d}	10	4 ^{1d}	4 ^{1d}	5	5 ^{1d}			
3	10	10	10	10	9	9	9	10	4	4	5	5			
4	10	10	10	10	8 ^{1d}	9	9	10	4	4	5	4 ^{1d}			
5	10	10	9 ^{1d}	9 ^{1d}	8	7 ^{2d}	8 ^{1d}	7 ^{3d}	2 ^{2d}	2 ^{2d}	2 ^{2d}	2 ^{2d}			
6	10	10	9	9	8	7	8	7	2	2	2	2			
7	10	10	9	9	8	7	7 ^{1d}	7	2	1 ^{1d}	1 ^{1d}	2			
*A = Pan weight (mg) Tray color code: <u>Grey</u> Analyst: <u>BL</u> Date: <u>02-22-23</u>															
*B = Pan + Larvae weight (mg) Analyst: <u>BL</u> Date: <u>03-16-23</u>															
C = Larvae weight (mg) = B - A Analyst: <u>h</u>															
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: <u>h</u>															
Average weight per initial number of larvae (mg)		Percent reduction from control (%)		0.771		-7.37		0.507		29.47		0.122		83.17	

*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: 100
Test dates: March 07-14, 2023

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	14.37	20.77	6.40	0.640	0.719	11.0	0.640	100.0	0.719	11.0	Not applicable
	B	10	10	14.82	23.08	8.26	0.826			0.826				
	C	10	10	14.84	21.69	6.85	0.685			0.685				
	D	10	10	13.44	20.68	7.24	0.724			0.724				
300	E	10	10	14.06	22.70	8.64	0.864	0.808	6.0	0.864	100.0	0.808	6.0	-12.5
	F	10	10	13.50	21.83	8.33	0.833			0.833				
	G	10	10	15.80	23.58	7.78	0.778			0.778				
	H	10	10	14.92	22.50	7.58	0.758			0.758				
450	I	10	10	13.18	20.17	6.99	0.699	0.729	3.8	0.699	100.0	0.729	3.8	-1.4
	J	10	10	14.38	21.63	7.25	0.725			0.725				
	K	10	10	13.94	21.20	7.26	0.726			0.726				
	L	10	10	13.99	21.65	7.66	0.766			0.766				
600	M	10	10	13.17	21.51	8.34	0.834	0.813	5.5	0.834	95.0	0.771	5.5	-7.3
	N	10	10	12.89	20.36	7.47	0.747			0.747				
	O	10	9	13.30	20.75	7.45	0.828			0.745				
	P	10	9	13.67	21.26	7.59	0.843			0.759				
750	Q	10	8	14.35	19.60	5.25	0.656	0.701	8.6	0.525	72.5	0.507	7.5	29.4
	R	10	7	14.63	20.14	5.51	0.787			0.551				
	S	10	7	14.96	19.84	4.88	0.697			0.488				
	T	10	7	13.79	18.44	4.65	0.664			0.465				
900	U	10	2	13.35	15.10	1.75	0.875	0.815	23.6	0.175	15.0	0.122	41.3	83.1
	V	10	1	13.34	14.39	1.05	1.050			0.105				
	W	10	1	12.81	13.41	0.60	0.600			0.060				
	X	10	2	14.21	15.68	1.47	0.735			0.147				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.000	0.0	0.000	0.0	0.000	0.0	100.0
	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: 0.0858
 PMSD: 11.9
 MSD = Minimum Significant Difference
 PMSD = Percent Minimum Significant Difference

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



Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date: 3/7/2023	Test ID: PpKICR	Sample ID: REF-Ref Toxicant
End Date: 3/14/2023	Lab ID: ETS-Envir. Testing Sol.	Sample Type: KCL-Potassium chloride
Sample Date:	Protocol: FWCHR-EPA-821-R-02-013	Test Species: PP-Pimephales promelas

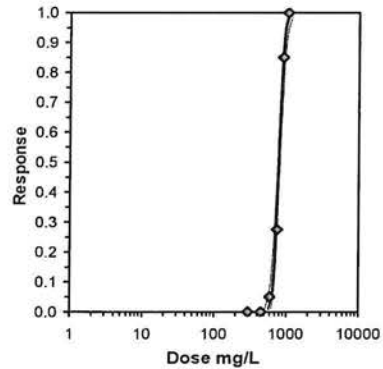
Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	0.9000	0.9000
750	0.8000	0.7000	0.7000	0.7000
900	0.2000	0.1000	0.1000	0.2000
1050	0.0000	0.0000	0.0000	0.0000

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	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.7250	0.7250	1.0202	0.9912	1.1071	5.685	4	10.00	10.00	11	40
*900	0.1500	0.1500	0.3927	0.3218	0.4636	20.862	4	10.00	10.00	34	40
1050	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

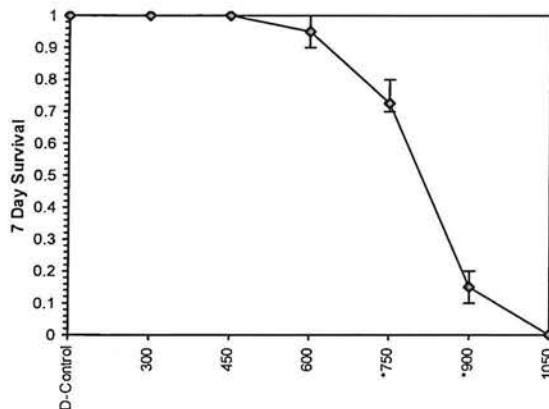
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.86486	0.884	0.22627	-0.3124
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

Parameter	Value	SE	95% Fiducial Limits		Maximum Likelihood-Probit						
			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter		
Slope	17.304	2.25651	12.8812	21.7267	0	3.56298	9.48773	0.46837	2.89658	0.05779	3
Intercept	-45.122	6.55635	-57.973	-32.272							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	578.288	512.636	623.059
EC05	3.355	633.181	577.563	671.447
EC10	3.718	664.543	614.996	699.301
EC15	3.964	686.576	641.282	719.115
EC20	4.158	704.607	662.689	735.577
EC25	4.326	720.453	681.348	750.301
EC40	4.747	761.98	728.989	790.609
EC50	5.000	788.106	757.5	817.765
EC60	5.253	815.127	785.435	847.675
EC75	5.674	862.111	830.281	904.051
EC80	5.842	881.499	847.654	928.68
EC85	6.036	904.649	867.758	958.907
EC90	6.282	934.642	893.008	999.15
EC95	6.645	980.936	930.726	1063.14
EC99	7.326	1074.05	1003.72	1196.93



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

Larval Fish Growth and Survival Test-7 Day Growth

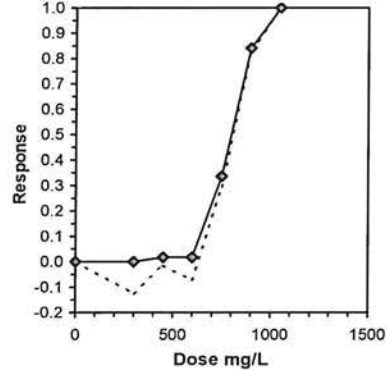
Start Date:	3/7/2023	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/14/2023	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	0.6400	0.8260	0.6850	0.7240
300	0.8640	0.8330	0.7780	0.7580
450	0.6990	0.7250	0.7260	0.7660
600	0.8340	0.7470	0.7450	0.7590
750	0.5250	0.5510	0.4880	0.4650
900	0.1750	0.1050	0.0600	0.1470
1050	0.0000	0.0000	0.0000	0.0000

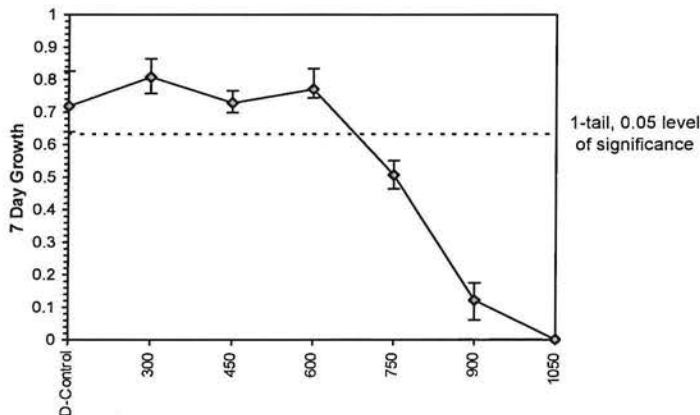
Conc-mg/L	Transform: Untransformed							1-Tailed			Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7188	1.0000	0.7188	0.6400	0.8260	11.035	4				0.7635	1.0000
300	0.8083	1.1245	0.8083	0.7580	0.8640	6.045	4	-2.389	2.290	0.0858	0.7635	1.0000
450	0.7290	1.0143	0.7290	0.6990	0.7660	3.793	4	-0.274	2.290	0.0858	0.7501	0.9825
600	0.7713	1.0730	0.7713	0.7450	0.8340	5.483	4	-1.401	2.290	0.0858	0.7501	0.9825
750	0.5073	0.7057	0.5073	0.4650	0.5510	7.537	4				0.5073	0.6644
900	0.1218	0.1694	0.1218	0.0600	0.1750	41.250	4				0.1218	0.1595
1050	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.95457	0.844	0.69954	0.40619						
Bartlett's Test indicates equal variances ($p = 0.40$)	2.91674	11.3449								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.0858	0.11937	0.00677	0.00281	0.11763	3, 12

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	615.32	66.09	281.00	632.97	-2.2740
IC10	638.89	10.41	603.52	661.12	-0.4848
IC15	662.47	10.51	627.16	689.20	-0.2870
IC20	686.05	10.94	650.40	717.28	-0.0800
IC25	709.62	11.65	674.16	745.36	0.1087
IC40	769.12	7.33	745.68	789.40	-0.0944
IC50	798.83	6.33	779.40	817.74	0.0711



Dose-Response Plot



Entered and Reviewed by
Jim Sumner
JS

Species: Pimephales promelas

PpKCICR Test Number: 100

Daily Chemistry:

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

Analyst		Day					
		(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
Concentration	Parameter	u	u	u	u	u	u
CONTROL, MHSW	pH (S.U.)	8.16	7.90	8.01	7.84	8.04	7.65
	Dissolved oxygen (mg/L)	7.8	7.9	7.9	7.4	7.6	7.2
	Conductivity (µmhos/cm)	290	290 ⁰³⁰¹²³	309		300	
	Alkalinity (mg CaCO ₃ /L)	92 (59)				59	
	Hardness (mg CaCO ₃ /L)	90 (92)				92	
	Temperature (°C)	24.8	24.4	24.7	24.5	24.6	24.8
300 mg KCl/L	pH (S.U.)	8.15	7.87	8.08	7.74	8.13	7.57
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.7	7.8	7.2
	Conductivity (µmhos/cm)	840		954		810	
	Temperature (°C)	24.8	24.6	24.6	24.8	24.6	24.5
450 mg KCl/L	pH (S.U.)	8.13	7.87	8.06	7.72	8.12	7.62
	Dissolved oxygen (mg/L)	7.9	7.9	7.9	7.7	7.8	7.2
	Conductivity (µmhos/cm)	1110		1110		1090	
	Temperature (°C)	24.9	24.6	24.6	24.8	24.8	24.7
600 mg KCl/L	pH (S.U.)	8.11	7.88	8.05	7.73	8.11	7.61
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.7	7.8	7.2
	Conductivity (µmhos/cm)	1340		1370		1310	
	Temperature (°C)	24.8	24.5	24.6	24.6	24.7	24.7
750 mg KCl/L	pH (S.U.)	8.10	7.89	8.04	7.74	8.11	7.60
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.7	7.9	7.2
	Conductivity (µmhos/cm)	1410		1450		1590	
	Temperature (°C)	24.8	24.6	24.6	24.6	24.7	24.7
900 mg KCl/L	pH (S.U.)	8.09	7.89	8.04	7.77	8.10	7.65
	Dissolved oxygen (mg/L)	7.8	7.9	8.0	7.6	7.9	7.2
	Conductivity (µmhos/cm)	1920		1910		1840	
	Temperature (°C)	24.8	24.6	24.6	24.6	24.7	24.8
1050 mg KCl/L	pH (S.U.)	8.11	7.88	8.04	7.88	8.11	7.68
	Dissolved oxygen (mg/L)	7.9	7.9	8.1	7.7	7.9	7.5
	Conductivity (µmhos/cm)	2200		2170		2090	
	Temperature (°C)	24.8	24.7	24.7	24.7	24.8	24.6
		Initial	Final	Initial	Final	Initial	Final

Species: *Pimephales promelas*

PpKCICR Test Number: 100

Concentration		Parameter	Day							
			(Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
			3		4		5		6	
Analyst		✓	BSL	BSL	BSL	BSL	BL ✓	BL ✓	✓	
CONTROL, MHSW	pH (S.U.)	7.92	7.83	8.16	7.75	8.07	7.90	8.04	7.90	
	Dissolved oxygen (mg/L)	7.4	7.4	7.8	7.4	7.9	7.6	7.9	7.5	
	Conductivity (µmhos/cm)	301		299		299		301		
	Alkalinity (mg CaCO ₃ /L)			62						
	Hardness (mg CaCO ₃ /L)			91						
	Temperature (°C)	24.7	24.6	24.7	25.0	24.7	24.7	24.7	24.6	
300 mg KCl/L	pH (S.U.)	8.05	7.83	8.18	7.71	8.10	7.88	8.16	7.90	
	Dissolved oxygen (mg/L)	7.6	7.4	7.9	7.3	8.0	7.4	7.8	7.4	
	Conductivity (µmhos/cm)	835		802		845		871		
	Temperature (°C)	24.7	24.5	24.8	24.8	24.7	24.5	24.7	24.4	
450 mg KCl/L	pH (S.U.)	8.04	7.86	8.18	7.68	8.12	7.88	8.15	7.88	
	Dissolved oxygen (mg/L)	7.7	7.4	8.0	7.2	8.0	7.3	7.8	7.3	
	Conductivity (µmhos/cm)	1080		1030		1100		1110		
	Temperature (°C)	24.7	24.5	24.7	24.8	24.8	24.5	24.8	24.4	
600 mg KCl/L	pH (S.U.)	8.02	7.83	8.18	7.71	8.12	7.87	8.14	7.87	
	Dissolved oxygen (mg/L)	7.8	7.4	8.0	7.1	8.0	7.3	7.8	7.4	
	Conductivity (µmhos/cm)	1340		1280		1380		1390		
	Temperature (°C)	24.8	24.7	24.7	24.7	24.8	24.4	24.8	24.6	
750 mg KCl/L	pH (S.U.)	8.02	7.86	8.17	7.67	8.12	7.81	8.13	7.88	
	Dissolved oxygen (mg/L)	7.8	7.4	8.0	7.1	8.0	7.3	7.8	7.4	
	Conductivity (µmhos/cm)	1590		1540		1650 (1650)		1630		
	Temperature (°C)	24.8	24.6	24.8	24.7	24.7	24.6	24.7	24.7	
900 mg KCl/L	pH (S.U.)	8.03	7.96	8.17	7.78	8.12	7.92	8.13	7.90	
	Dissolved oxygen (mg/L)	7.9	7.5	8.0	7.1	8.0	7.4	7.9	7.3	
	Conductivity (µmhos/cm)	1850		1800		1930 (1930)		1890		
	Temperature (°C)	24.8	24.6	24.8	24.9	24.7	24.5	24.8	24.7	
1050 mg KCl/L	pH (S.U.)	8.04	7.98	8.16	7.77	8.10	7.86	8.13	7.89	
	Dissolved oxygen (mg/L)	8.0	7.5	8.0	7.2	8.0	7.6	7.9	7.3	
	Conductivity (µmhos/cm)	2130		2060		2110 (2110)		2190		
	Temperature (°C)	24.8	24.6	24.8	24.7	24.8	24.5	24.8	24.4	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	

* wrote on wrong line BSL 03-12-23