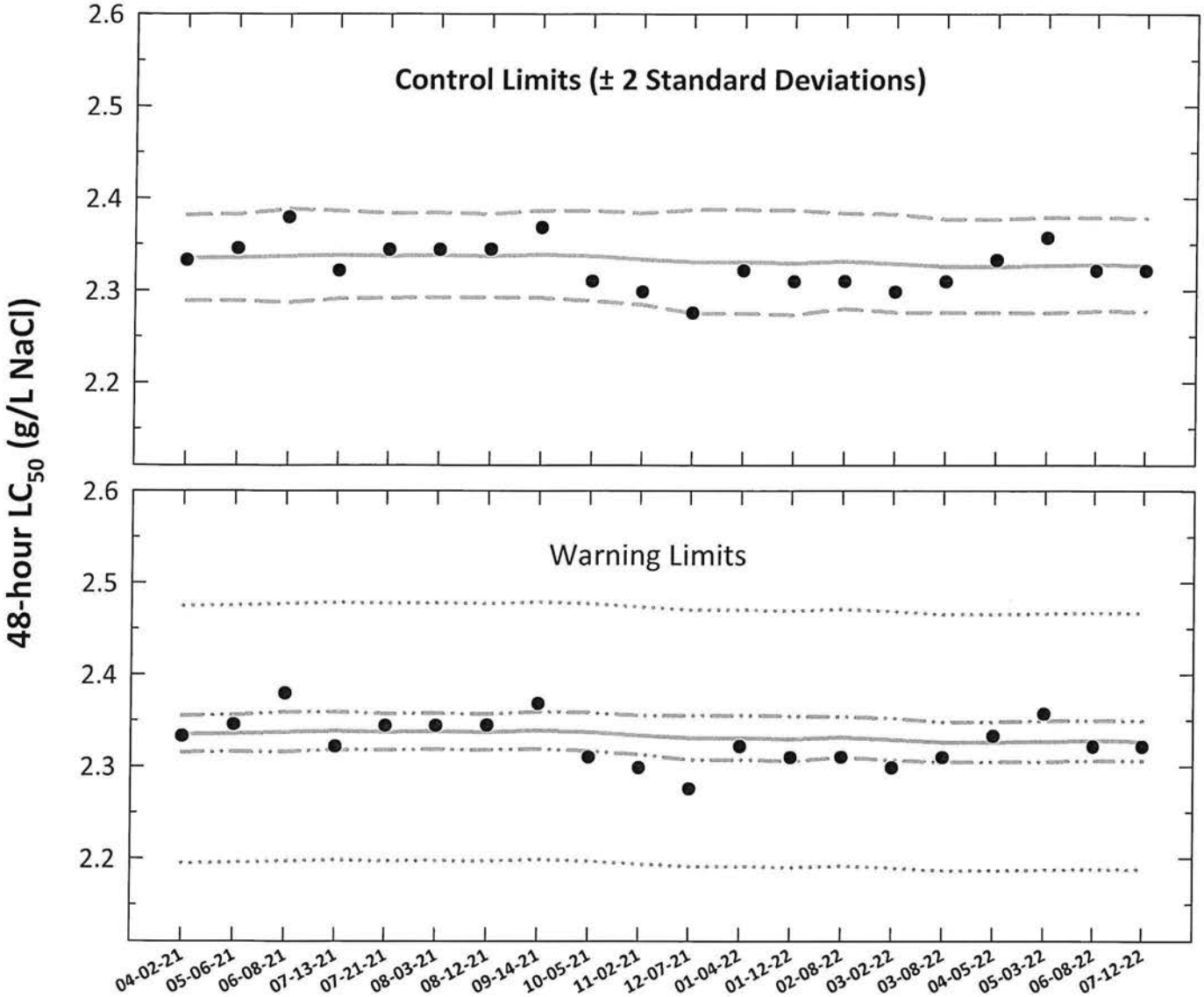
Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	04-02-21	2.3330	0.3679	0.3683	0.0043	2.3350	2.2889	2.3820	2.3153	2.3552	2.1949	2.4751
2	05-06-21	2.3455	0.3702	0.3684	0.0044	2.3356	2.2892	2.3829	2.3157	2.3558	2.1955	2.4757
3	06-08-21	2.3795	0.3765	0.3687	0.0047	2.3373	2.2872	2.3885	2.3159	2.3592	2.1971	2.4775
4	07-13-21	2.3217	0.3658	0.3689	0.0044	2.3385	2.2912	2.3868	2.3182	2.3591	2.1982	2.4788
5	07-21-21	2.3442	0.3700	0.3687	0.0043	2.3373	2.2918	2.3837	2.3179	2.3572	2.1971	2.4776
6	08-03-21	2.3442	0.3700	0.3688	0.0043	2.3379	2.2923	2.3843	2.3184	2.3577	2.1976	2.4781
7	08-12-21	2.3449	0.3701	0.3687	0.0042	2.3373	2.2924	2.3831	2.3181	2.3569	2.1971	2.4775
8	09-14-21	2.3682	0.3744	0.3690	0.0044	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
9	10-05-21	2.3099	0.3636	0.3687	0.0046	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
10	11-02-21	2.2985	0.3614	0.3681	0.0046	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 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.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 382

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 #: 2107

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	M	TG	M
Control, MHSW	pH (S.U.)	7.79	7.67	7.67
	Dissolved oxygen (mg/L)	7.6	7.7	7.7
	Conductivity (µmhos/cm)	293		
	Alkalinity (mg/L $CaCO_3$)	59		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	25.0	25.2	25.1
1750 mg/L	pH (S.U.)	7.77	7.79	7.80
	Dissolved oxygen (mg/L)	7.9	7.9	7.8
	Conductivity (µmhos/cm)	3260		
	Temperature (°C)	25.1	24.9	25.3
2000 mg/L	pH (S.U.)	7.78	7.80	7.82
	Dissolved oxygen (mg/L)	7.9	8.0	7.8
	Conductivity (µmhos/cm)	3660		
	Temperature (°C)	24.9	24.9	25.0
2250 mg/L	pH (S.U.)	7.77	7.80	7.84
	Dissolved oxygen (mg/L)	7.9	8.2	7.8
	Conductivity (µmhos/cm)	4090		
	Temperature (°C)	24.9	24.8	25.0
2500 mg/L	pH (S.U.)	7.77	7.79	7.85
	Dissolved oxygen (mg/L)	8.0	8.1	7.9
	Conductivity (µmhos/cm)	4470		
	Temperature (°C)	24.9	25.0	24.9
2750 mg/L	pH (S.U.)	7.77	7.80	7.84
	Dissolved oxygen (mg/L)	8.0	8.1	7.9
	Conductivity (µmhos/cm)	4890		
	Temperature (°C)	25.0	24.8	25.0

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664685

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 382

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	07-12-22	* 0530	JL	0800	JL	2B3	BLACK	07-07-22A
24	07-13-22			0800	JL			
48 Termination	07-14-22			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):	07-05-22 ~~B~~ C 07-07-22 JL
Age:	< 24-hours old
Date and time organisms were born between:	07-11-22 1528 TO 07-12-22 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.06
	Temperature (°C): 25.1

Survival Data (number of living organisms):

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	4 d	5	5	5	3 d	3 d	3 d	3 d
48 Termination	4 d	2 d	2 d	3 d	1 d	1 d	2 d	1 d	0	0	0	0
Mean Survival	55%				25%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2246.1
Upper 95% confidence limit (mg NaCl/L)	2394.5
48-hour LC_{50} (mg NaCl/L)	2321.2

Comments:

Test Reviewed by: JL

Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	7/12/2022	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	7/14/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.8000	0.4000	0.4000	0.6000
2500	0.2000	0.2000	0.4000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5500	0.5500	0.8407	0.6847	1.1071	23.960	4	10.00	10.00	9	20
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

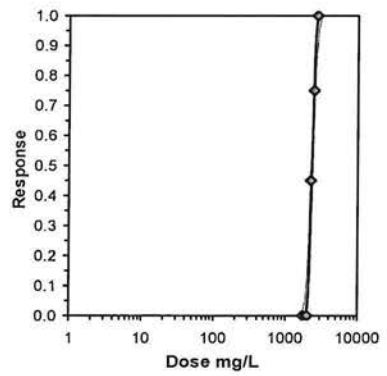
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77333	0.868	1.18991	3.75354
Equality of variance cannot be confirmed				

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

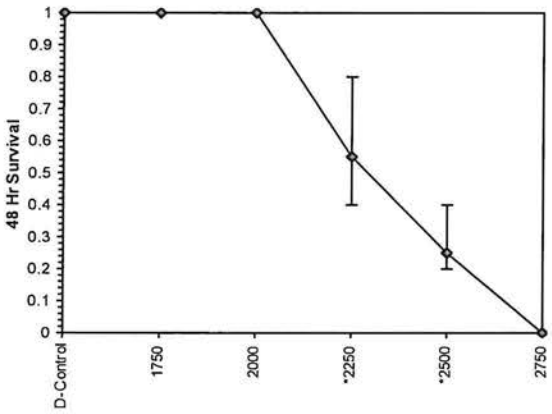
Maximum Likelihood-Probit

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

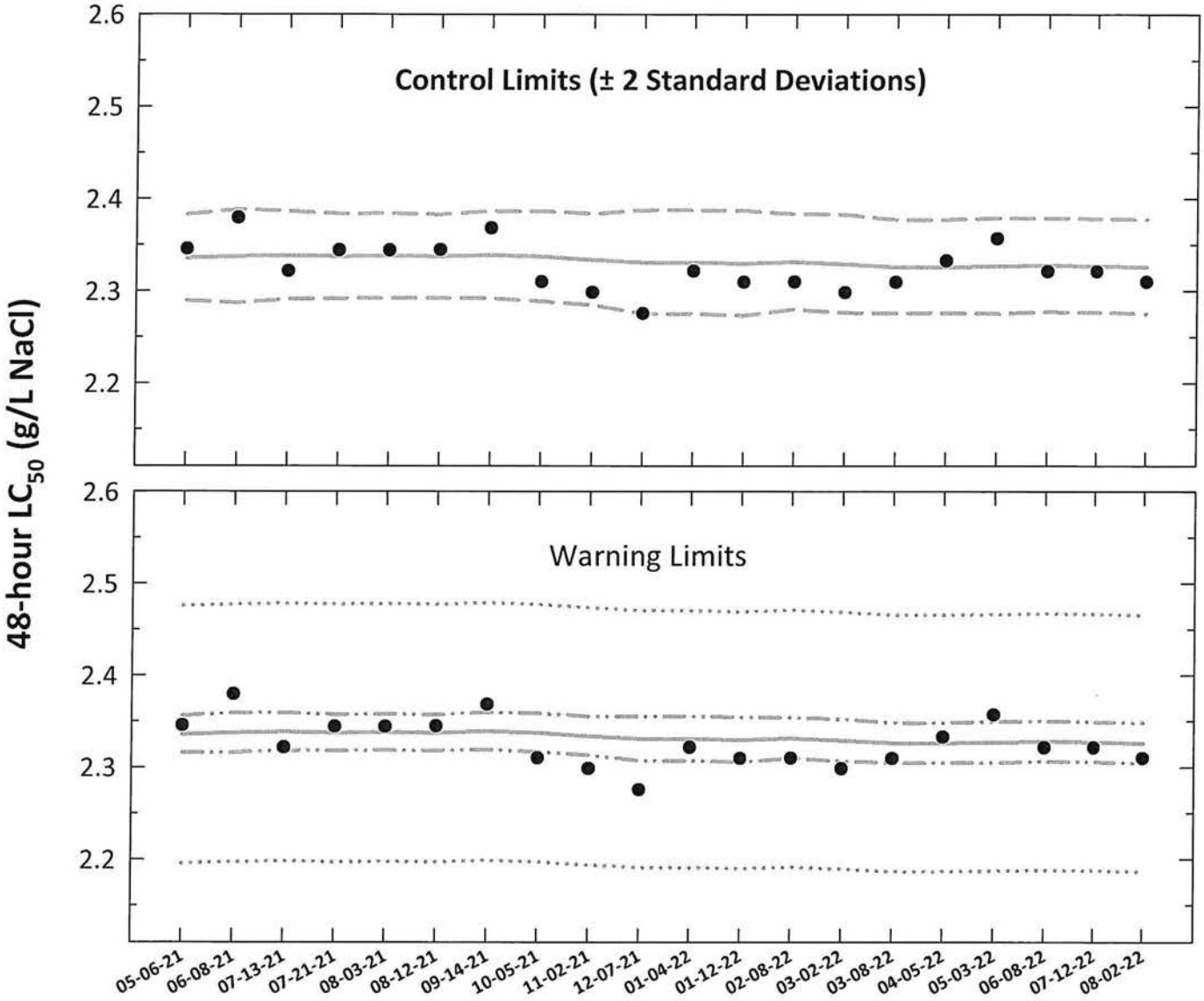
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1937.95	1738.22	2048.06
EC05	3.355	2043.15	1882.24	2134.37
EC10	3.718	2101.55	1962.46	2183.38
EC15	3.964	2141.89	2017.55	2218.14
EC20	4.158	2174.51	2061.62	2247.05
EC25	4.326	2202.88	2099.4	2273.01
EC40	4.747	2276.03	2192.77	2345.02
EC50	5.000	2321.21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



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	05-06-21	2.3455	0.3702	0.3684	0.0044	2.3356	2.2892	2.3829	2.3157	2.3558	2.1955	2.4757
2	06-08-21	2.3795	0.3765	0.3687	0.0047	2.3373	2.2872	2.3885	2.3159	2.3592	2.1971	2.4775
3	07-13-21	2.3217	0.3658	0.3689	0.0044	2.3385	2.2912	2.3868	2.3182	2.3591	2.1982	2.4788
4	07-21-21	2.3442	0.3700	0.3687	0.0043	2.3373	2.2918	2.3837	2.3179	2.3572	2.1971	2.4776
5	08-03-21	2.3442	0.3700	0.3688	0.0043	2.3379	2.2923	2.3843	2.3184	2.3577	2.1976	2.4781
6	08-12-21	2.3449	0.3701	0.3687	0.0042	2.3373	2.2924	2.3831	2.3181	2.3569	2.1971	2.4775
7	09-14-21	2.3682	0.3744	0.3690	0.0044	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
8	10-05-21	2.3099	0.3636	0.3687	0.0046	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
9	11-02-21	2.2985	0.3614	0.3681	0.0046	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 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.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 383

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 #: 2107

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
	Analyst	EC	K	EC
Control, MHSW	pH (S.U.)	7.06	7.33	7.56
	Dissolved oxygen (mg/L)	7.6	7.9	7.7
	Conductivity (µmhos/cm)	300		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	25.1	25.2	25.0
1750 mg/L	pH (S.U.)	7.63	7.33	7.71
	Dissolved oxygen (mg/L)	7.8	8.0	7.7
	Conductivity (µmhos/cm)	3430		
	Temperature (°C)	25.0	25.0	25.2
2000 mg/L	pH (S.U.)	7.69	7.38	7.73
	Dissolved oxygen (mg/L)	7.8	7.9	7.7
	Conductivity (µmhos/cm)	3810		
	Temperature (°C)	25.0	25.0	25.2
2250 mg/L	pH (S.U.)	7.71	7.40	~~7.75~~ 08-04-22 7.75
	Dissolved oxygen (mg/L)	7.8	7.9	7.8
	Conductivity (µmhos/cm)	4180		
	Temperature (°C)	25.2	25.1	25.1
2500 mg/L	pH (S.U.)	7.74	7.45	7.75
	Dissolved oxygen (mg/L)	7.8	7.9	7.9
	Conductivity (µmhos/cm)	4610		
	Temperature (°C)	25.0	24.8	25.3
2750 mg/L	pH (S.U.)	7.77	7.55	7.76
	Dissolved oxygen (mg/L)	7.8	7.9	7.9
	Conductivity (µmhos/cm)	5050		
	Temperature (°C)	25.0	24.8	25.1

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664685

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*
EPA-821-R-02-012, Method 2002.0

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 383

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	08-02-22	*0540	JL	0745	JL	283	GREEN	07-28-22B
24	08-03-22			0740	JL			
48 Termination	08-04-22			0742	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):	07-26-22 D
Age:	< 24-hours old
Date and time organisms were born between:	08-01-22 1432 TO 08-02-22 0540
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.55
	Temperature (°C): 25.1

Survival Data (number of living organisms):

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

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

Comment codes: d = dead, u = unhealthy

Statistics:

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

Comments:

Test Reviewed by: JL

Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	8/2/2022	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/4/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.6000	0.2000	0.6000	0.8000
2500	0.2000	0.2000	0.0000	0.4000
2750	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5500	0.5500	0.8357	0.4636	1.1071	32.195	4	10.00	10.00	9	20
*2500	0.2000	0.2000	0.4594	0.2255	0.6847	40.823	4	10.00	10.00	16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

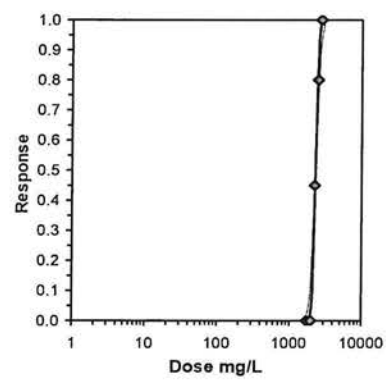
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.70445	0.868	-0.8618	4.01856
Equality of variance cannot be confirmed				

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

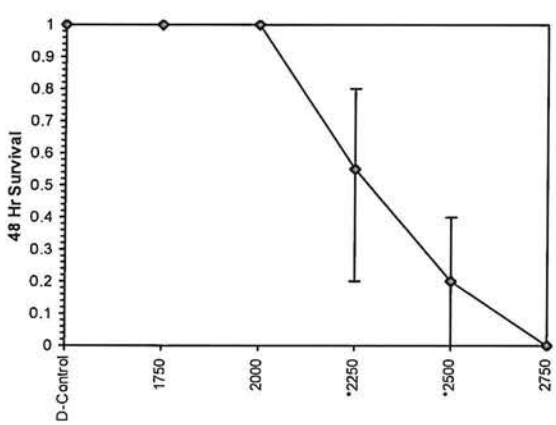
Maximum Likelihood-Probit

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

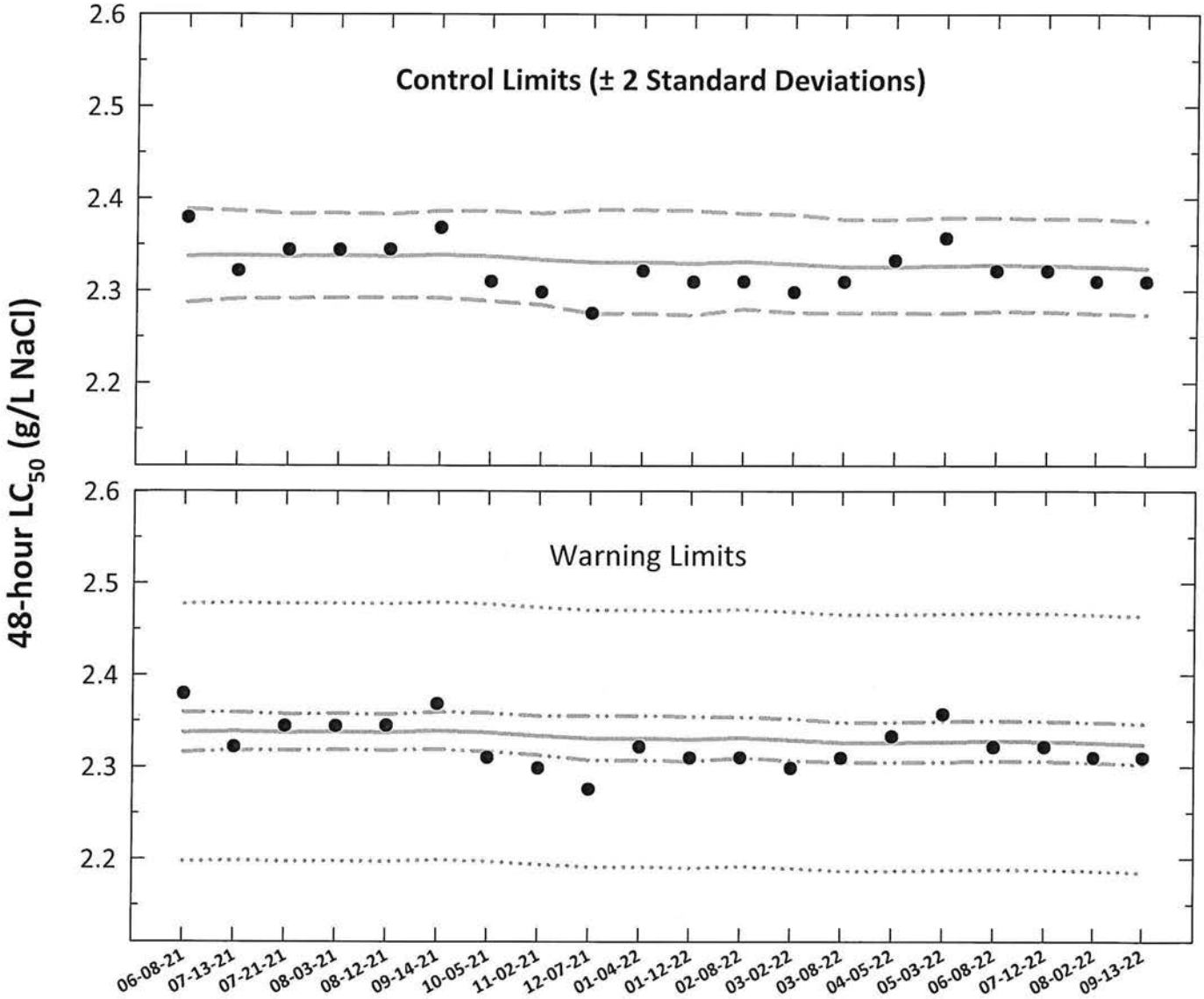
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1948.47	1752	2054.85
EC05	3.355	2048.07	1890.23	2136.13
EC10	3.718	2103.23	1966.98	2182.25
EC15	3.964	2141.28	2019.59	2214.95
EC20	4.158	2172.02	2061.61	2242.15
EC25	4.326	2198.74	2097.59	2266.57
EC40	4.747	2267.53	2186.32	2334.36
EC50	5.000	2309.94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



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	06-08-21	2.3795	0.3765	0.3687	0.0047	2.3373	2.2872	2.3885	2.3159	2.3592	2.1971	2.4775
2	07-13-21	2.3217	0.3658	0.3689	0.0044	2.3385	2.2912	2.3868	2.3182	2.3591	2.1982	2.4788
3	07-21-21	2.3442	0.3700	0.3687	0.0043	2.3373	2.2918	2.3837	2.3179	2.3572	2.1971	2.4776
4	08-03-21	2.3442	0.3700	0.3688	0.0043	2.3379	2.2923	2.3843	2.3184	2.3577	2.1976	2.4781
5	08-12-21	2.3449	0.3701	0.3687	0.0042	2.3373	2.2924	2.3831	2.3181	2.3569	2.1971	2.4775
6	09-14-21	2.3682	0.3744	0.3690	0.0044	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
7	10-05-21	2.3099	0.3636	0.3687	0.0046	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
8	11-02-21	2.2985	0.3614	0.3681	0.0046	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 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.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 3&4

Dilution Preparation:

Test concentrations (mg/L NaCl)	1750	2000	2250	2500	2750
mL Stock solution	3.5	4.0	4.5	5.0	5.5
mL Dilution water (MHSW)	196.5	196.0	195.5	195.0	194.5
Total volume (mL)	200	200	200	200	200

A stock solution was prepared by diluting 10 g NaCl into 100 mL deionized water. This 100,000 mg/L NaCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2130

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	EC	EC N	EC
Control, MHSW	pH (S.U.)	7.20	7.41	7.38
	Dissolved oxygen (mg/L)	7.9	8.0	7.9
	Conductivity (µmhos/cm)	309		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	87		
	Temperature (°C)	25.0	25.0	25.2
1750 mg/L	pH (S.U.)	7.54	7.59	7.63
	Dissolved oxygen (mg/L)	7.9	8.0	7.9
	Conductivity (µmhos/cm)	3320		
	Temperature (°C)	24.9	25.2	25.1
2000 mg/L	pH (S.U.)	7.60	7.62	7.66
	Dissolved oxygen (mg/L)	8.0	8.0	8.0
	Conductivity (µmhos/cm)	3850		
	Temperature (°C)	24.9	24.9	25.1
2250 mg/L	pH (S.U.)	7.63	7.63	7.66
	Dissolved oxygen (mg/L)	8.0	8.0	8.0
	Conductivity (µmhos/cm)	4250		
	Temperature (°C)	25.1	24.9	25.2
2500 mg/L	pH (S.U.)	7.63	7.63	7.70
	Dissolved oxygen (mg/L)	8.0	8.1	8.1
	Conductivity (µmhos/cm)	4670		
	Temperature (°C)	25.1	24.9	25.2
2750 mg/L	pH (S.U.) 09.13.22 EC	~~7.63~~ 7.69	7.66	7.70
	Dissolved oxygen (mg/L)	8.0	8.0	8.1
	Conductivity (µmhos/cm)	5060		
	Temperature (°C)	25.1	25.0	25.0

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066485

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 384

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	09-13-22	*0745	[illegible]	0956	[illegible]	2B3	RCD	09-06-22B
24	09-14-22			0955	[illegible]			
48 Termination	09-15-22			0957	[illegible]			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	09-06-22 D
Age:	< 24-hours old
Date and time organisms were born between:	09-12-22 1500 TO 09-13-22 0513
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.82
	Temperature (°C): 25.1

Survival Data (number of living organisms):

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

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

Comment codes: d = dead, u = unhealthy

Statistics:

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

Comments:

Test Reviewed by: [illegible]

Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	9/13/2022	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/15/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.2000	0.8000	0.6000	0.4000
2500	0.2000	0.4000	0.2000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5000	0.5000	0.7854	0.4636	1.1071	35.048	4	10.00	10.00	10	20
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

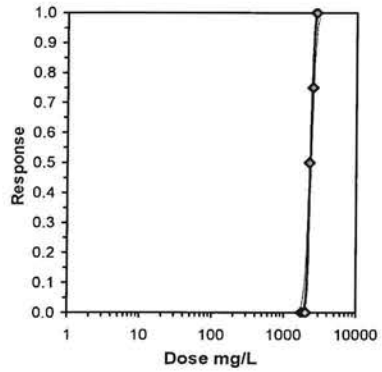
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77068	0.868	0.14468	4.85271
Equality of variance cannot be confirmed				

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

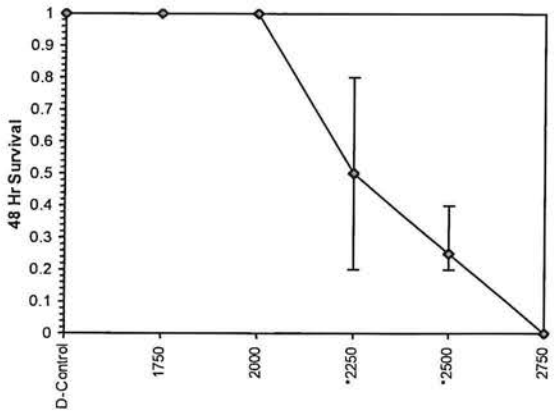
Maximum Likelihood-Probit

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

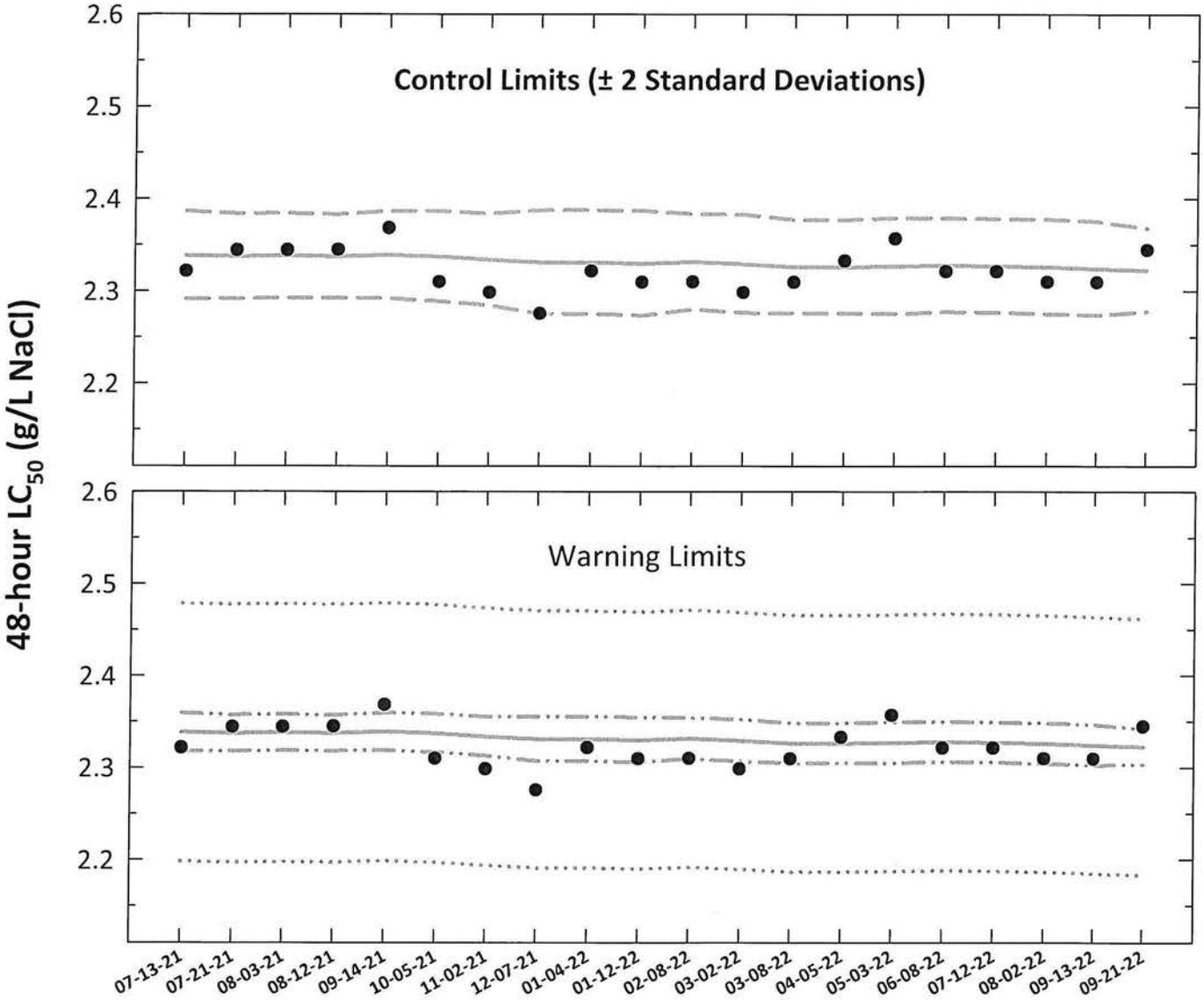
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1918.7	1718.88	2029.87
EC05	3.355	2025.8	1864.5	2117.99
EC10	3.718	2085.31	1945.73	2168.05
EC15	3.964	2126.45	2001.56	2203.56
EC20	4.158	2159.72	2046.25	2233.09
EC25	4.326	2188.68	2084.59	2259.6
EC40	4.747	2263.39	2179.46	2333.09
EC50	5.000	2309.55	2233.77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



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	07-13-21	2.3217	0.3658	0.3689	0.0044	2.3385	2.2912	2.3868	2.3182	2.3591	2.1982	2.4788
2	07-21-21	2.3442	0.3700	0.3687	0.0043	2.3373	2.2918	2.3837	2.3179	2.3572	2.1971	2.4776
3	08-03-21	2.3442	0.3700	0.3688	0.0043	2.3379	2.2923	2.3843	2.3184	2.3577	2.1976	2.4781
4	08-12-21	2.3449	0.3701	0.3687	0.0042	2.3373	2.2924	2.3831	2.3181	2.3569	2.1971	2.4775
5	09-14-21	2.3682	0.3744	0.3690	0.0044	2.3390	2.2921	2.3869	2.3190	2.3595	2.1987	2.4794
6	10-05-21	2.3099	0.3636	0.3687	0.0046	2.3373	2.2888	2.3868	2.3165	2.3584	2.1970	2.4775
7	11-02-21	2.2985	0.3614	0.3681	0.0046	2.3338	2.2847	2.3839	2.3127	2.3553	2.1937	2.4738
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 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.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 385

Dilution Preparation:

Test concentrations (mg/L NaCl)	1750	2000	2250	2500	2750
mL Stock solution	3.5	4.0	4.5	5.0	5.5
mL Dilution water (MHSW)	196.5	196.0	195.5	195.0	194.5
Total volume (mL)	200	200	200	200	200

A stock solution was prepared by diluting 10 g NaCl into 100 mL deionized water. This 100,000 mg/L NaCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2130

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
		EC M	M	EC
Control, MHSW	pH (S.U.)	7.50	7.51	7.48
	Dissolved oxygen (mg/L)	7.6	7.6	7.9
	Conductivity (µmhos/cm)	314		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	87		
	Temperature (°C)	25.0	25.2	25.1
1750 mg/L	pH (S.U.)	7.64	7.74	7.67
	Dissolved oxygen (mg/L)	7.6	7.6	8.0
	Conductivity (µmhos/cm)	3490		
	Temperature (°C)	25.1	25.0	25.0
2000 mg/L	pH (S.U.)	7.67	7.75	7.72
	Dissolved oxygen (mg/L)	7.6	7.5	8.0
	Conductivity (µmhos/cm)	3860		
	Temperature (°C)	25.1	25.0	25.0
2250 mg/L	pH (S.U.)	7.68	7.75	7.73
	Dissolved oxygen (mg/L)	7.6	7.5	7.9
	Conductivity (µmhos/cm)	4340		
	Temperature (°C)	24.9	25.1	25.0
2500 mg/L	pH (S.U.)	7.68	7.76	7.75
	Dissolved oxygen (mg/L)	7.6	7.5	8.0
	Conductivity (µmhos/cm)	4660		
	Temperature (°C)	24.9	25.1	24.9
2750 mg/L	pH (S.U.)	7.69	7.76	7.77
	Dissolved oxygen (mg/L)	7.6	7.5	8.0
	Conductivity (µmhos/cm)	5200		
	Temperature (°C)	24.9	25.0	25.1

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066468S

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Ceriodaphnia dubia*

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 385

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	09-21-22	*0500	H	0700	H	282	BLAIK	09-15-22B
24	09-22-22			0700	H			
48 Termination	09-23-22			0700	H			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	09-13-22 A
Age:	< 24-hours old
Date and time organisms were born between:	09-20-22 1500 TO 09-21-22 0500
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.82
	Temperature (°C): 25.0

Survival Data (number of living organisms)*:*

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	5	5	5	5	4 1d	4 1d	3 2d	4 1d
48 Termination	2 3d	4 1d	4 1d	3 2d	2 3d	1 4d	1 4d	1 4d	0 4d	0 4d	0 3d	0 4d
Mean Survival	65%				25%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2271.6
Upper 95% confidence limit (mg NaCl/L)	2416.3
48-hour LC_{50} (mg NaCl/L)	2344.9

Comments:

Test Reviewed by: H

Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	9/21/2022	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/23/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
1750	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2250	0.4000	0.8000	0.8000	0.6000
2500	0.4000	0.2000	0.2000	0.2000
2750	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.6500	0.6500	0.9463	0.6847	1.1071	21.467	4	10.00	10.00	7	20
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

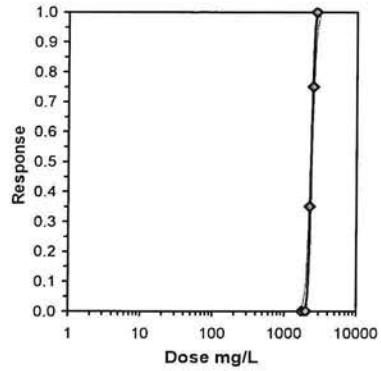
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.76164	0.868	-0.432	3.36576
Equality of variance cannot be confirmed				

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

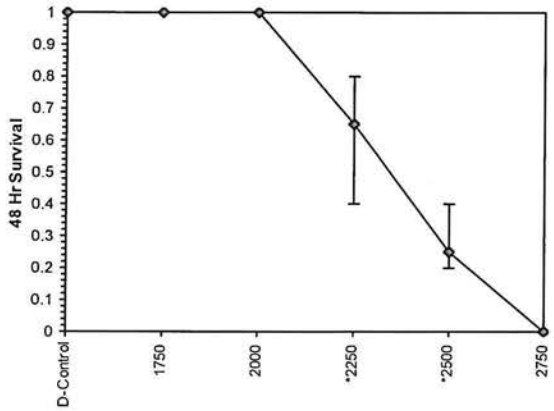
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	31.7967	5.91059	20.2119	43.3815	0	1.4914	7.81472	0.68426	3.37012	0.03145	5
Intercept	-102.16	19.935	-141.23	-63.086							
TSCR											

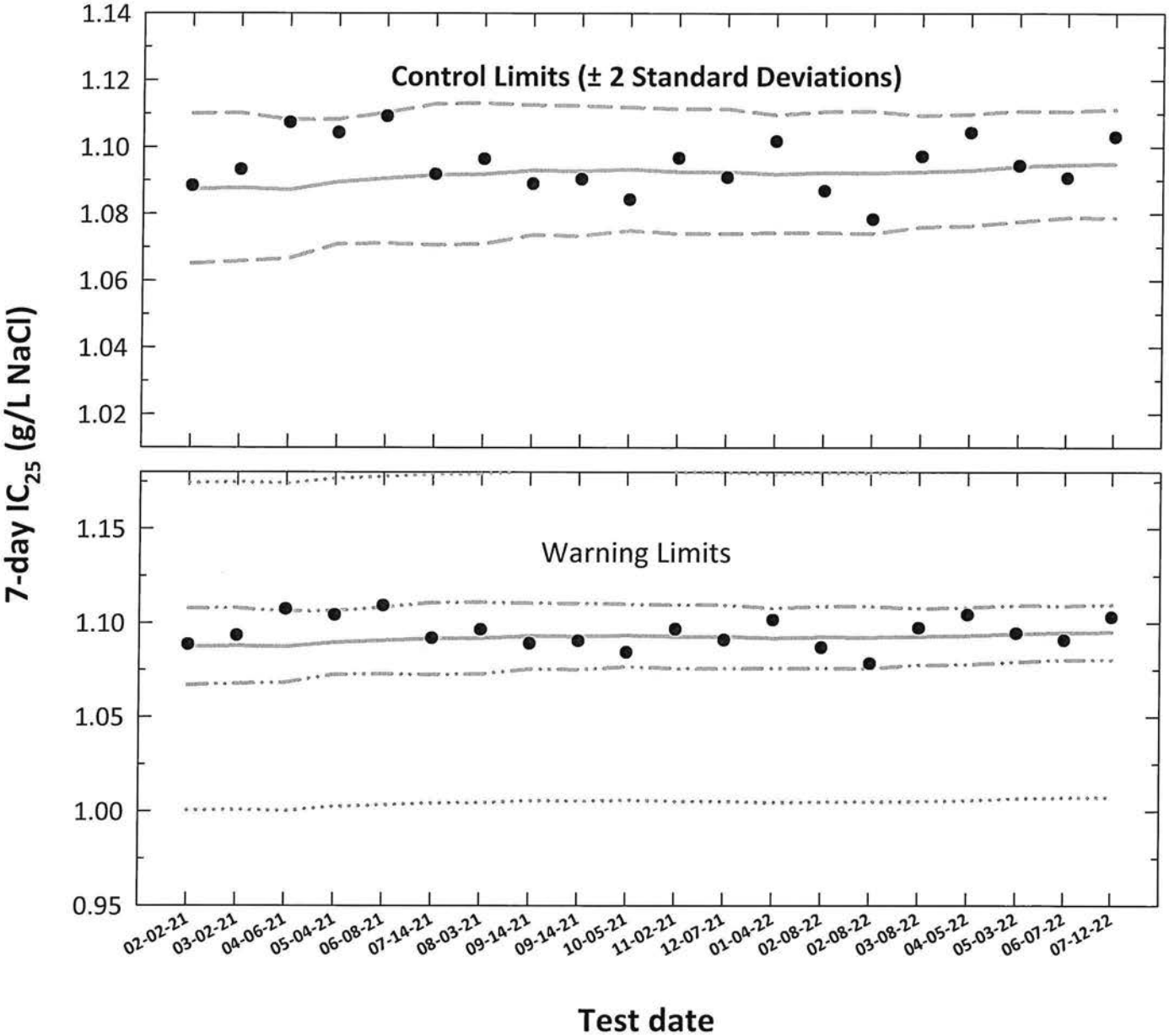
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1981.32	1782.02	2088.57
EC05	3.355	2081.56	1921.73	2170.17
EC10	3.718	2137.05	1999.28	2216.47
EC15	3.964	2175.32	2052.43	2249.29
EC20	4.158	2206.22	2094.86	2276.61
EC25	4.326	2233.09	2131.18	2301.14
EC40	4.747	2302.24	2220.69	2369.31
EC50	5.000	2344.87	2271.62	2416.29
EC60	5.253	2388.29	2319.33	2468.85
EC75	5.674	2462.24	2391.58	2568.72
EC80	5.842	2492.23	2418.34	2612.2
EC85	6.036	2527.63	2448.59	2665.24
EC90	6.282	2572.9	2485.68	2735.18
EC95	6.645	2641.49	2539.52	2844.65
EC99	7.326	2775.12	2639.56	3066.72



Dose-Response Plot



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



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

Independent Review by Kelley E. Keenan:

Entered and Reviewed by Jim Sumner

Independent Review by Kelley E. Keenan:

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	02-02-21	1.0884	0.0406	0.0364	0.0045	1.0873	1.0651	1.1100	1.0669	1.1077	1.0003	1.1743
2	03-02-21	1.0933	0.0368	0.0365	0.0044	1.0878	1.0659	1.1101	1.0677	1.1079	1.0008	1.1748
3	04-06-21	1.1074	0.0387	0.0363	0.0041	1.0872	1.0666	1.1082	1.0683	1.1061	1.0002	1.1742
4	05-04-21	1.1043	0.0443	0.0372	0.0037	1.0895	1.0710	1.1083	1.0725	1.1065	1.0024	1.1767
5	06-08-21	1.1092	0.0431	0.0376	0.0039	1.0906	1.0712	1.1103	1.0728	1.1083	1.0033	1.1778
6	07-14-21	1.0919	0.0450	0.0381	0.0042	1.0917	1.0707	1.1130	1.0725	1.1108	1.0043	1.1790
7	08-03-21	1.0964	0.0382	0.0382	0.0042	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

Warning Limits = Mean logarithmic IC_{25} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

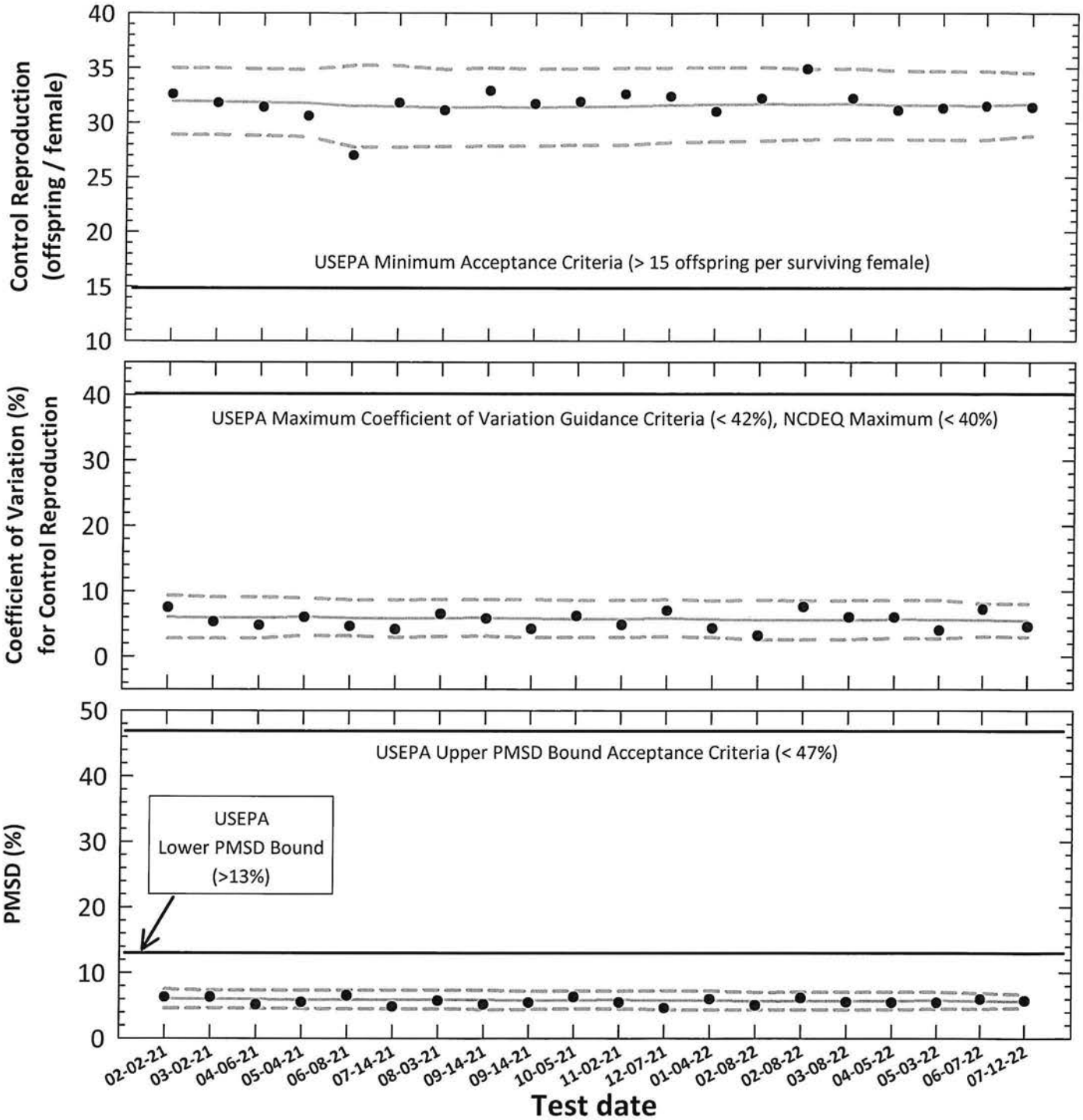
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

Central Tendency (mean Control Reproduction, CV or PMSD)

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

Independent Review by Kelley E. Keenan:

Entered and Reviewed by Jim Sumner

Independent Review by Kelley E. Keenan:

ETS Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination: Control Survival (%)	ToxCal Determination: Control Reproduction Mean (offspring/female)	ToxCal Determination: Control Reproduction CV (%)	ToxCal Determination: Test MSD	ToxCal Determination: Test PMSD (%)	Control Reproduction (offspring/female) CT	Control Reproduction 95% Confidence Interval CT - 2S	Control Reproduction 95% Confidence Interval CT + 2S	Control Reproduction CV (%) CT	Control Reproduction CV 95% Confidence Interval CT - 2S	Control Reproduction CV 95% Confidence Interval CT + 2S	Test PMSD (%) CT	Test PMSD 95% Confidence Interval CT - 2S	Test PMSD 95% Confidence Interval CT + 2S
1	02-02-21	100	32.6	7.5	2.045	6.3	31.9	28.9	35.0	6.0	2.8	9.3	6.0	4.6	7.5
2	03-02-21	100	31.8	5.3	2.004	6.3	31.9	28.9	35.0	5.9	2.7	9.1	6.0	4.7	7.3
3	04-06-21	100	31.4	4.8	1.615	5.1	31.9	28.8	34.9	5.9	2.8	9.1	6.0	4.6	7.4
4	05-04-21	100	30.6	6.0	1.690	5.5	31.8	28.7	34.9	6.1	3.2	8.9	5.9	4.5	7.3
5	06-08-21	100	27.0	4.6	1.762	6.5	31.5	27.8	35.2	5.9	3.1	8.7	5.9	4.5	7.4
6	07-14-21	100	31.8	4.1	1.546	4.9	31.5	27.8	35.2	5.8	2.9	8.6	5.9	4.5	7.4
7	08-03-21	100	31.1	6.5	1.785	5.7	31.3	27.8	34.8	5.9	3.1	8.7	5.9	4.5	7.4
8	09-14-21	100	32.9	5.8	1.698	5.2	31.4	27.9	35.0	5.9	3.1	8.7	5.9	4.4	7.3
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	4.4	7.0
18	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0
19	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
20	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

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.

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 273

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2101				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	07-12-22 0530 TO 0810									
Culture board:	07-05-22 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	2	3	5	10	11	12	18	19	23	25
Transfer vessel information:	pH (S.U.): 8.06 Temperature (°C): 25.1									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

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

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	07-12-22	0827	07-01-22	06-30-22	07-07-22A	JH
1	07-13-22	0740	↓	↓	↓	JH
2	07-14-22	0736	↓	↓	07-07-22C	JH
3	07-15-22	0734	↓	↓	↓	JH
4	07-16-22	0825	↓	↓	07-12-22A	JH
5	07-17-22	0735	↓	↓	↓	JH
6	07-18-22	0736	↓	↓	↓	JH
7	07-19-22	0730				JH

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

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312562
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312562
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312562
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% of Male Adults:	0%	≤ 20%	**7-day LC_{50}** (mg/L NaCl)	1400
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	31.4	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	4.6%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1103.0

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 273**

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	3	4	4	5	4	4	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	13	13	10	12	10	12	12	13	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	17	13	18	16	15	16	14	16	17
Total young produced		30	33	30	32	33	29	32	31	33	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd 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:	0%
Mean Offspring/Female:	31.4

600 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	6	4	3	4	5	4	5	5	5	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	13	10	13	13	11	12	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	18	18	16	18	14	17	17	16	17
Total young produced		31	34	34	30	36	31	33	34	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:	0%
Mean Offspring/Female:	32.6
% Reduction from Control:	-3.8%

Independent Review by Kelley E. Keenan:

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 273**

800 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	5	4	5	5	6	4	3	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	10	13	12	13	13	10	11	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	17	18	15	17	13	14	14	14	18
Total young produced		31	35	32	33	34	32	31	27	28	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:	0%
Mean Offspring/Female:	31.6
% Reduction from Control:	-0.6%

1000 mg NaCl/L ***Survival and Reproduction Data***

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.8
% Reduction from Control:	1.9%

Independent Review by Kelley E. Keenan:

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 273**

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.6
% Reduction from Control:	43.9%

1400 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	0	2	1	1	0	1	3	1	1	2
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	2	0	0	0	3	1	0	1	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	0	0	0	0	0	0	0
Total young produced		2	2	1	1	3	2	3	2	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:	0%
Mean Offspring/Female:	1.9
% Reduction from Control:	93.9%

Independent Review by Kelley E. Keenan: K

Independent Review by Kelley E. Keenan:

ETS
Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	4	4	5	4	4	5	4	4	41
5	11	13	13	10	12	10	12	12	13	10	116
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	13	18	16	15	16	14	16	17	157
Total	30	33	30	32	33	29	32	31	33	31	314

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	6	4	3	4	5	4	5	5	5	3	44
5	10	12	13	10	13	13	11	12	12	10	116
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	18	16	18	14	17	17	16	17	166
Total	31	34	34	30	36	31	33	34	33	30	326

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	4	5	5	6	4	3	3	4	43
5	10	13	10	13	12	13	13	10	11	11	116
6	0	0	0	0	0	0	0	0	0	0	0
7	17	17	18	15	17	13	14	14	14	18	157
Total	31	35	32	33	34	32	31	27	28	33	316

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	5	3	3	3	4	4	5	5	41
5	12	10	12	13	10	13	11	13	10	12	116
6	0	0	0	0	0	0	0	0	0	0	0
7	15	13	16	15	15	13	17	15	17	15	151
Total	32	27	33	31	28	29	32	32	32	32	308

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	2	4	3	3	2	1	4	3	3	3	28
5	9	6	10	8	6	5	7	6	9	5	71
6	0	0	0	0	0	0	0	0	0	0	0
7	7	10	6	5	10	11	5	9	5	9	77
Total	18	20	19	16	18	17	16	18	17	17	176

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	2	1	1	0	1	3	1	1	2	12
5	2	0	0	0	3	1	0	1	0	0	7
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	2	2	1	1	3	2	3	2	1	2	19

Entered and Reviewed by Jim Sumner

Independent Review by Kelley E. Keenan:

Environmental Testing Solutions, Inc.

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 #273

Test dates: July 12-19, 2022

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	30	33	30	32	33	29	32	31	33	31	100	31.4	4.6	Not applicable
600	31	34	34	30	36	31	33	34	33	30	100	32.6	6.2	-3.8
800	31	35	32	33	34	32	31	27	28	33	100	31.6	7.9	-0.6
1000	32	27	33	31	28	29	32	32	32	32	100	30.8	6.6	1.9
1200	18	20	19	16	18	17	16	18	17	17	100	17.6	7.2	43.9
1400	2	2	1	1	3	2	3	2	1	2	100	1.9	38.8	93.9

Dunnett's MSD value: 1.804

PMSD: 5.7

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	7/12/2022	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	7/19/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	30.000	33.000	30.000	32.000	33.000	29.000	32.000	31.000	33.000	31.000
600	31.000	34.000	34.000	30.000	36.000	31.000	33.000	34.000	33.000	30.000
800	31.000	35.000	32.000	33.000	34.000	32.000	31.000	27.000	28.000	33.000
1000	32.000	27.000	33.000	31.000	28.000	29.000	32.000	32.000	32.000	32.000
1200	18.000	20.000	19.000	16.000	18.000	17.000	16.000	18.000	17.000	17.000
1400	2.000	2.000	1.000	1.000	3.000	2.000	3.000	2.000	1.000	2.000

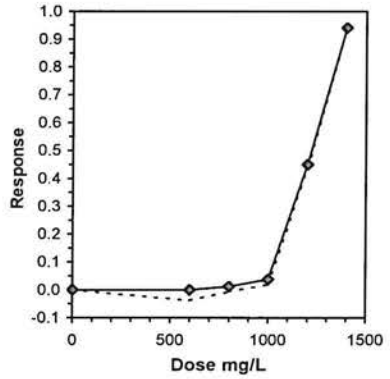
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	31.400	1.0000	31.400	29.000	33.000	4.554	10				32.000	1.0000
600	32.600	1.0382	32.600	30.000	36.000	6.169	10	-1.521	2.287	1.804	32.000	1.0000
800	31.600	1.0064	31.600	27.000	35.000	7.922	10	-0.253	2.287	1.804	31.600	0.9875
1000	30.800	0.9809	30.800	27.000	33.000	6.636	10	0.760	2.287	1.804	30.800	0.9625
*1200	17.600	0.5605	17.600	16.000	20.000	7.187	10	17.489	2.287	1.804	17.600	0.5500
*1400	1.900	0.0605	1.900	1.000	3.000	38.835	10	37.387	2.287	1.804	1.900	0.0594

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.96989	1.035	-0.5215	0.241
Bartlett's Test indicates equal variances (p = 0.02)	13.5787	15.0863		

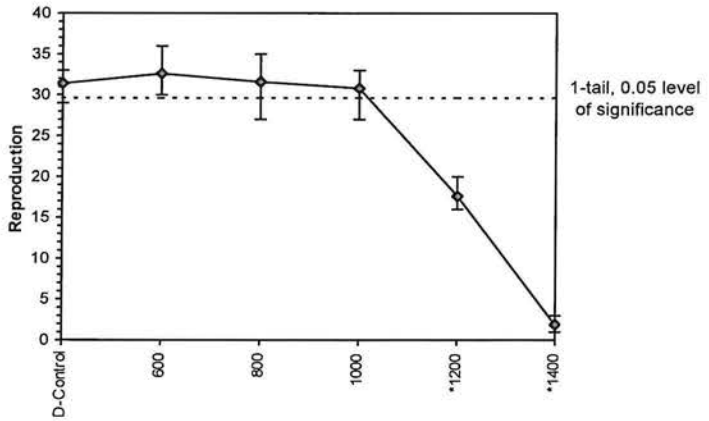
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.80428	0.05746	1522.98	3.11296	1.2E-43	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1006.06	49.2249	799.375	1020.7	-2.6560
IC10	1030.3	8.52773	1011.31	1043.27	-0.2390
IC15	1054.55	7.72081	1038.02	1066.57	-0.1843
IC20	1078.79	7.0318	1063.77	1089.62	-0.1075
IC25	1103.03	6.4983	1089.04	1113.43	-0.0076
IC40	1175.76	6.18483	1164.54	1187.81	0.2908
IC50	1220.38	4.91018	1211.25	1229.71	0.0526



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Independent Review by Kelley E. Keenan:

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 273**

Daily Chemistry:

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

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	K	K	K	K	K	K
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.39	7.59	7.39	7.60	7.49	7.56
	Dissolved oxygen (mg/L)	7.6	7.6	7.9	7.8	7.6	7.6
	Conductivity (µmhos/cm)	293		302		313	
	Alkalinity (mg $CaCO_3$/L)	59				59	
	Hardness (mg $CaCO_3$/L)	86				86	
	Temperature (°C)	24.8	25.2	24.8	25.1	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.63	7.62	7.59	7.67	7.69	7.66
	Dissolved oxygen (mg/L)	7.6	7.6	7.8	7.7	7.6	7.6
	Conductivity (µmhos/cm)	1360		1390		1410	
	Temperature (°C)	24.9	24.9	24.8	25.3	25.0	24.9
800 mg NaCl/L	pH (S.U.)	7.65	7.63	7.59	7.74	7.73	7.72
	Dissolved oxygen (mg/L)	7.6	7.7	7.8	7.7	7.6	7.5
	Conductivity (µmhos/cm)	1710		1720		1780	
	Temperature (°C)	24.9	24.9	24.9	25.0	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.67	7.64	7.60	7.74	7.74	7.73
	Dissolved oxygen (mg/L)	7.6	7.7	7.8	7.7	7.6	7.5
	Conductivity (µmhos/cm)	2050		2090		2140	
	Temperature (°C)	25.0	25.1	24.9	25.3	24.9	25.0
1200 mg NaCl/L	pH (S.U.)	7.71	7.63	7.61	7.74	7.75	7.73
	Dissolved oxygen (mg/L)	7.6	7.7	7.8	7.8	7.7	7.5
	Conductivity (µmhos/cm)	2400		2430		2470	
	Temperature (°C)	25.0	24.8	24.8	25.2	25.0	25.0
1400 mg NaCl/L	pH (S.U.)	7.71	7.64	7.65	7.78	7.77	7.75
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.8	7.7	7.6
	Conductivity (µmhos/cm)	2730		2750		2800	
	Temperature (°C)	25.0	25.1	24.8	25.2	25.0	25.1
		Initial	Final	Initial	Final	Initial	Final

Independent Review by Kelley E. Keenan: K

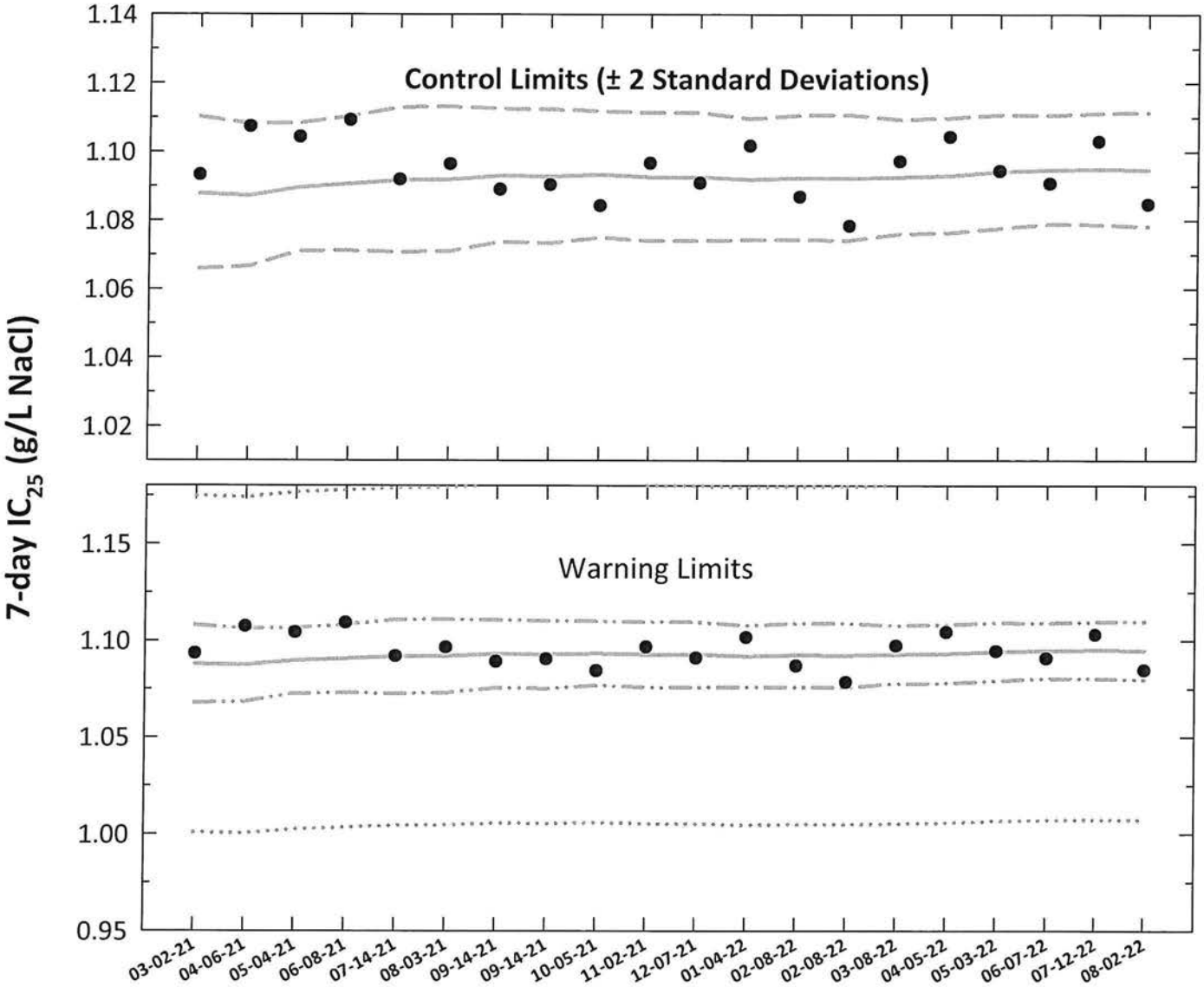
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 273**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	K	BSL	BSL	BSL	BSL	TG	TG	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.36	7.54	7.31	7.52	7.30	7.55	7.60	7.55
	Dissolved oxygen (mg/L)	7.6	7.7	7.7	7.6	7.7	7.5	7.7 ~~7.5~~	7.8
	Conductivity (µmhos/cm)	298		294		303		310	
	Alkalinity (mg $CaCO_3$/L)			59					
	Hardness (mg $CaCO_3$/L)	K 07-19-22		86				K 07-19-22	
	Temperature (°C)	24.9	24.9	24.9	25.1	24.8	24.9	24.7	25.2
600 mg NaCl/L	pH (S.U.)	7.68	7.77	7.78	7.76	7.65	7.65	7.69	7.56
	Dissolved oxygen (mg/L)	7.6	7.6	7.7	7.6	7.7	7.6	7.7	7.9
	Conductivity (µmhos/cm)	1360		1380		1440		1450	
	Temperature (°C)	24.7	25.1	24.9	24.8	24.9	25.1	24.8	25.0
800 mg NaCl/L	pH (S.U.)	7.71	7.83	7.83	7.79	7.72	7.73	7.70	7.59
	Dissolved oxygen (mg/L)	7.6	7.6	7.8	7.6	7.7	7.5	7.6	7.9
	Conductivity (µmhos/cm)	1720		1690		1760		1790	
	Temperature (°C)	24.9	25.2	24.8	24.8	24.9	25.1	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.75	7.83	7.85	7.79	7.73	7.71	7.75	7.60
	Dissolved oxygen (mg/L)	7.6	7.5	7.8	7.6	7.7	7.6	7.7	7.8
	Conductivity (µmhos/cm)	2080		2010		2110		2150	
	Temperature (°C)	24.9	25.0	24.8	25.0	25.0	25.0	24.9	25.0
1200 mg NaCl/L	pH (S.U.)	7.76	7.86	7.86	7.81	7.77	7.75	7.77	7.61
	Dissolved oxygen (mg/L)	7.6	7.4	7.8	7.6	7.7	7.7	7.6	7.8
	Conductivity (µmhos/cm)	2420		2350		2490	TG 07-18-22	~~2150~~ 2510	
	Temperature (°C)	24.8	24.9	24.8	25.0	25.0	25.0	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	7.70	7.86	7.88	7.81	7.79	7.78	7.81	7.61
	Dissolved oxygen (mg/L)	7.6	~~7.0~~ 07-18-22 ~~8.0~~ BSL	7.9	7.6	7.7	7.5	7.7	7.9
	Conductivity (µmhos/cm)	2750		2720		2830		2880 ~~2510~~	TG 07-18-22
	Temperature (°C)	24.8	25.1	24.8	24.9	25.0	25.0	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Independent Review by Kelley E. Keenan: K

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} ± 2 standard deviations converted to anti-logarithmic values)
- **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	03-02-21	1.0933	0.0368	0.0365	0.0044	1.0878	1.0659	1.1101	1.0677	1.1079	1.0008	1.1748
2	04-06-21	1.1074	0.0387	0.0363	0.0041	1.0872	1.0666	1.1082	1.0683	1.1061	1.0002	1.1742
3	05-04-21	1.1043	0.0443	0.0372	0.0037	1.0895	1.0710	1.1083	1.0725	1.1065	1.0024	1.1767
4	06-08-21	1.1092	0.0431	0.0376	0.0039	1.0906	1.0712	1.1103	1.0728	1.1083	1.0033	1.1778
5	07-14-21	1.0919	0.0450	0.0381	0.0042	1.0917	1.0707	1.1130	1.0725	1.1108	1.0043	1.1790
6	08-03-21	1.0964	0.0382	0.0382	0.0042	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

Warning Limits = Mean logarithmic IC_{25} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

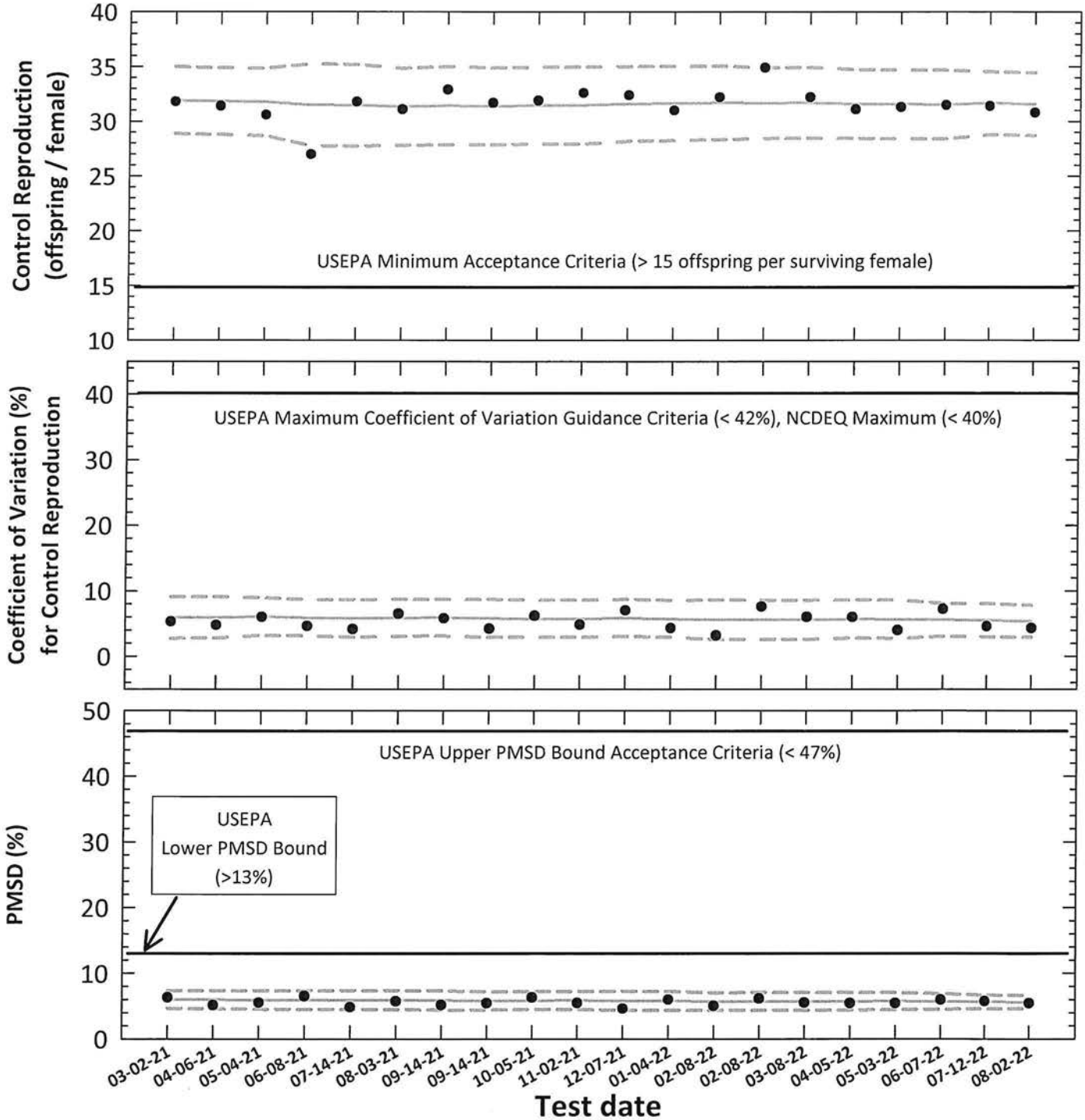
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control Survival	Control Reproduction		Test										
			Mean	CV	MSD	PMSD	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
		(%)	(offspring/female)	(%)		(%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	03-02-21	100	31.8	5.3	2.004	6.3	31.9	28.9	35.0	5.9	2.7	9.1	6.0	4.7	7.3
2	04-06-21	100	31.4	4.8	1.615	5.1	31.9	28.8	34.9	5.9	2.8	9.1	6.0	4.6	7.4
3	05-04-21	100	30.6	6.0	1.690	5.5	31.8	28.7	34.9	6.1	3.2	8.9	5.9	4.5	7.3
4	06-08-21	100	27.0	4.6	1.762	6.5	31.5	27.8	35.2	5.9	3.1	8.7	5.9	4.5	7.4
5	07-14-21	100	31.8	4.1	1.546	4.9	31.5	27.8	35.2	5.8	2.9	8.6	5.9	4.5	7.4
6	08-03-21	100	31.1	6.5	1.785	5.7	31.3	27.8	34.8	5.9	3.1	8.7	5.9	4.5	7.4
7	09-14-21	100	32.9	5.8	1.698	5.2	31.4	27.9	35.0	5.9	3.1	8.7	5.9	4.4	7.3
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	4.4	7.0
17	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0
18	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
19	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
20	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

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.

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 274

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2107				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	08-02-22 0540 TO 0800									
Culture board:	07-26-22 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	5	6	7	12	13	17	18	19	20	23
Transfer vessel information:	pH (S.U.): 7.55 Temperature (°C): 25.1									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

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

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	08-02-22	0811	07-28-22	07-28-22	07-29-22B	JL
1	08-03-22	0751	↓	↓	↓	JL
2	08-04-22	0805	↓	↓	07-29-22D	JL
3	08-05-22	0732	↓	↓	↓	JL
4	08-06-22	0841	↓	↓	07-29-22E	JL
5	08-07-22	0830	↓	↓	↓	JL
6	08-08-22	0736	↓	↓	↓	JL
7	08-09-22	0714				JL

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

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312562
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312562
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312562
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% of Male Adults:	0%	≤ 20%	**7-day LC_{50}** (mg/L NaCl)	>1400
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	30.8	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	4.37%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1084.7

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 274**

CONTROL

Survival and Reproduction Data

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	4	5	3	5	5	5	5	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	12	12	12	10	12	11	11	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	15	14	12	16	17	12	14	16	16
Total young produced		33	31	30	29	31	32	29	30	32	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd 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:	0%
Mean Offspring/Female:	30.8

600 mg NaCl/L

Survival and Reproduction Data

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	4	5	4	6	5	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	14	13	10	12	12	13	10	13	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	13	16	17	17	14	17	19	15	13	18
Total young produced		32	34	31	34	30	36	34	33	30	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:	0%
Mean Offspring/Female:	32.9
% Reduction from Control:	-6.8%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 274**

800 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	3	5	6	5	5	5	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	10	12	10	12	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	16	15	14	18	15	15	15	18	13
Total young produced		31	30	30	32	33	32	32	31	34	30
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.5
% Reduction from Control:	-2.3%

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	6	4	3	5	5	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	9	11	12	10	10	10	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	12	14	14	11	16	12	14	13	14	14
Total young produced		28	30	29	26	31	27	29	28	30	30
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	28.8
% Reduction from Control:	6.5%

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 274**

1200 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	3	4	5	3	5	4	4	3	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	7	9	5	9	6	10	8	5	8	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	5	6	10	5	4	4	4	8	5	7
Total young produced		15	18	19	19	13	19	16	17	16	20
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.2
% Reduction from Control:	44.2%

1400 mg NaCl/L ***Survival and Reproduction Data***

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2.9
% Reduction from Control:	80.6%

Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	4	5	3	5	5	5	5	3	45
5	13	11	12	12	12	10	12	11	11	12	116
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	14	12	16	17	12	14	16	16	147
Total	**33**	**31**	**30**	**29**	**31**	**32**	**29**	**30**	**32**	**31**	**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	5	4	5	4	6	5	5	5	5	49
5	14	13	10	12	12	13	10	13	12	12	121
6	0	0	0	0	0	0	0	0	0	0	0
7	13	16	17	17	14	17	19	15	13	18	159
Total	**32**	**34**	**31**	**34**	**30**	**36**	**34**	**33**	**30**	**35**	**329**

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	6	5	5	5	4	4	4	46
5	12	11	10	12	10	12	12	12	12	13	116
6	0	0	0	0	0	0	0	0	0	0	0
7	14	16	15	14	18	15	15	15	18	13	153
Total	**31**	**30**	**30**	**32**	**33**	**32**	**32**	**31**	**34**	**30**	**315**

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	6	4	3	5	5	5	4	4	44
5	12	12	9	11	12	10	10	10	12	12	110
6	0	0	0	0	0	0	0	0	0	0	0
7	12	14	14	11	16	12	14	13	14	14	134
Total	**28**	**30**	**29**	**26**	**31**	**27**	**29**	**28**	**30**	**30**	**288**

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	3	4	5	3	5	4	4	3	3	37
5	7	9	5	9	6	10	8	5	8	10	77
6	0	0	0	0	0	0	0	0	0	0	0
7	5	6	10	5	4	4	4	8	5	7	58
Total	**15**	**18**	**19**	**19**	**13**	**19**	**16**	**17**	**16**	**20**	**172**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	2	3	1	3	4	3	2	2	3	25
5	1	2	0	0	0	0	1	0	0	0	4
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	**3**	**4**	**3**	**1**	**3**	**4**	**4**	**2**	**2**	**3**	**29**

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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 #274

Test dates: August 02-09, 2022

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	33	31	30	29	31	32	29	30	32	31	100	30.8	4.3	Not applicable
600	32	34	31	34	30	36	34	33	30	35	100	32.9	6.3	-6.8
800	31	30	30	32	33	32	32	31	34	30	100	31.5	4.3	-2.3
1000	28	30	29	26	31	27	29	28	30	30	100	28.8	5.4	6.5
1200	15	18	19	19	13	19	16	17	16	20	100	17.2	12.8	44.2
1400	3	4	3	1	3	4	4	2	2	3	100	2.9	34.3	90.6

Dunnett's MSD value: 1.676

PMSD: 5.4

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	8/2/2022	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	8/9/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	31.000	30.000	29.000	31.000	32.000	29.000	30.000	32.000	31.000
600	32.000	34.000	31.000	34.000	30.000	36.000	34.000	33.000	30.000	35.000
800	31.000	30.000	30.000	32.000	33.000	32.000	32.000	31.000	34.000	30.000
1000	28.000	30.000	29.000	26.000	31.000	27.000	29.000	28.000	30.000	30.000
1200	15.000	18.000	19.000	19.000	13.000	19.000	16.000	17.000	16.000	20.000
1400	3.000	4.000	3.000	1.000	3.000	4.000	4.000	2.000	2.000	3.000

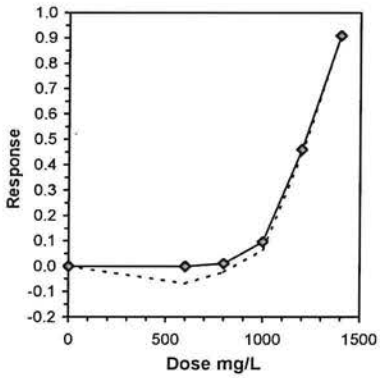
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	30.800	1.0000	30.800	29.000	33.000	4.275	10				31.850	1.0000
600	32.900	1.0682	32.900	30.000	36.000	6.319	10	-2.865	2.287	1.676	31.850	1.0000
800	31.500	1.0227	31.500	30.000	34.000	4.298	10	-0.955	2.287	1.676	31.500	0.9890
*1000	28.800	0.9351	28.800	26.000	31.000	5.379	10	2.728	2.287	1.676	28.800	0.9042
*1200	17.200	0.5584	17.200	13.000	20.000	12.797	10	18.552	2.287	1.676	17.200	0.5400
*1400	2.900	0.0942	2.900	1.000	4.000	34.291	10	38.059	2.287	1.676	2.900	0.0911

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.85326	1.035	-0.311	-0.2623
Bartlett's Test indicates equal variances (p = 0.19)	7.49321	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		1.67631	0.05443	1392.38	2.68704	2.5E-44	5, 54
Treatments vs D-Control										

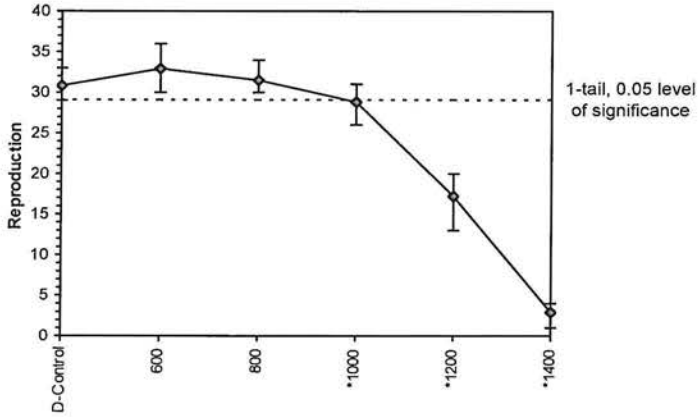
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	892.037	31.5223	825.246	954.196	0.0908
IC10	1002.33	19.3364	940.35	1019.47	-1.3437
IC15	1029.78	8.05917	1014.14	1045.85	-0.0617
IC20	1057.24	7.68673	1041.32	1072.33	-0.0032
IC25	1084.7	7.7733	1068.39	1098.97	0.0554
IC40	1167.07	10.3757	1149.3	1186.36	0.1119
IC50	1217.83	9.16147	1199.47	1231.41	-0.3038



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

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 274**

Daily Chemistry:

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

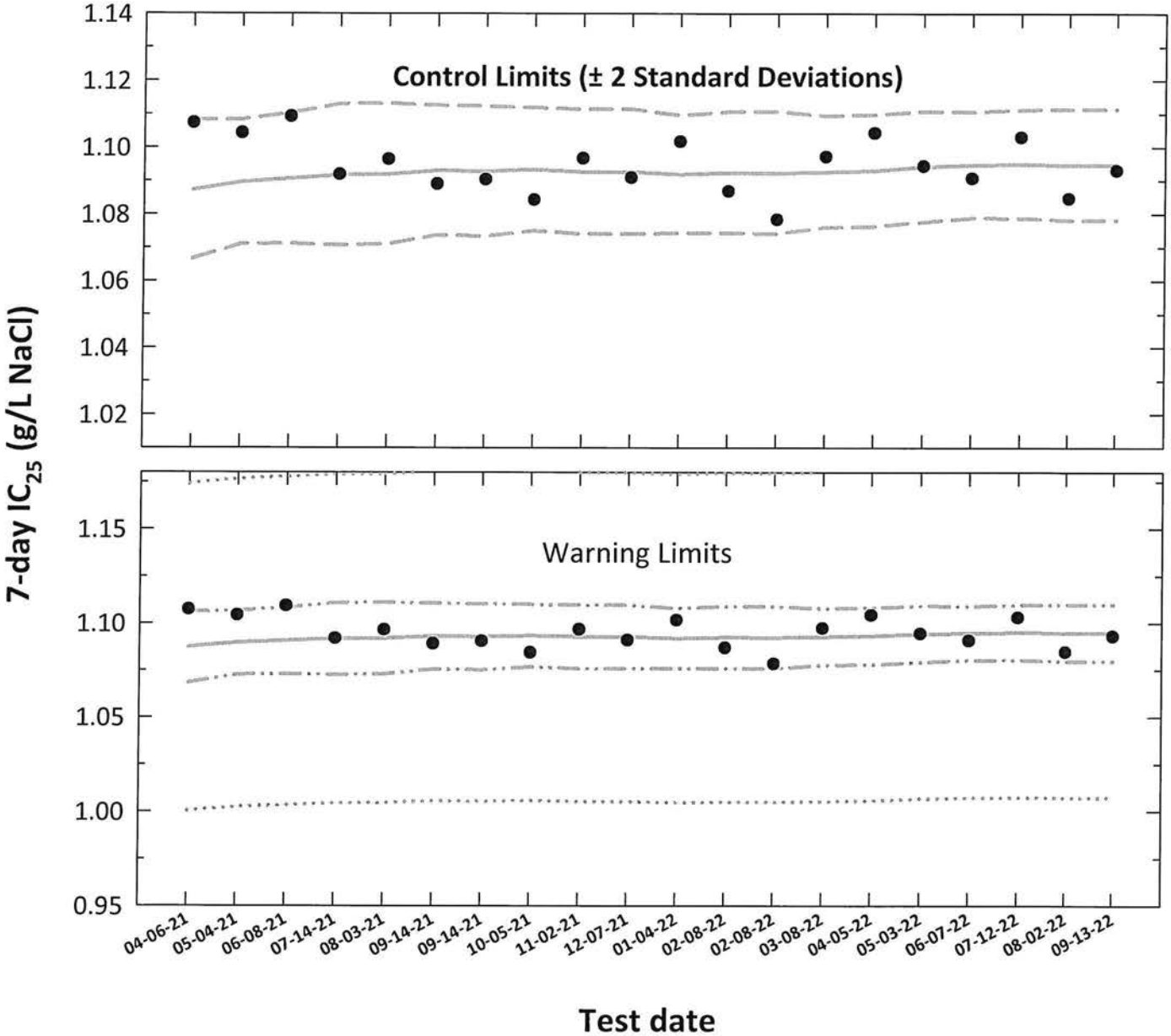
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	EC	EC N	EC N	EC	EC	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.06	7.52	7.13	7.63	7.23	7.53
	Dissolved oxygen (mg/L)	~~6.30~~ 08.02.22 EC 7.6	7.6	7.5	7.9	7.7	7.6
	Conductivity (µmhos/cm)	300	08.03.22 EC	~~1420~~ 314		300	
	Alkalinity (mg $CaCO_3$/L)	60				60	
	Hardness (mg $CaCO_3$/L)	86		N 08.03.22		90	
	Temperature (°C)	25.0	25.1	24.7	25.1	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.44	7.54	7.45	7.63	7.55	7.58
	Dissolved oxygen (mg/L)	~~6.44~~ 08.02.22 7.7	7.6	7.5	7.9	7.8	7.7
	Conductivity (µmhos/cm)	1390		~~1780~~ 08.03.22 EC 1420		1420	
	Temperature (°C)	24.9	24.9	24.8	24.8	24.8	25.0
800 mg NaCl/L	pH (S.U.)	7.51	7.53	7.51	7.64	7.61	7.61
	Dissolved oxygen (mg/L)	7.7	7.6	7.6	7.8	7.8	7.8
	Conductivity (µmhos/cm)	1760		~~2150~~ 08.03.22 EC 1780		1800	
	Temperature (°C)	24.9	24.9	24.7	25.2	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.56	7.55	7.53	7.64	7.66	7.62
	Dissolved oxygen (mg/L)	7.7	7.6	7.6	7.9	7.8	7.8
	Conductivity (µmhos/cm)	2120		2150		2180	
	Temperature (°C)	25.0	25.0	24.7	24.9	24.9	24.9
1200 mg NaCl/L	pH (S.U.)	7.56	7.56	7.60	7.65	7.67	7.62
	Dissolved oxygen (mg/L)	7.7	7.6	7.6	7.9	7.8	7.8
	Conductivity (µmhos/cm)	2480		2510		2540	
	Temperature (°C)	25.0	24.9	24.8	24.9	25.0	24.9
1400 mg NaCl/L	pH (S.U.) 08.02.22 EC	~~7.56~~ 7.63	7.56	7.62	7.66	7.69	7.64
	Dissolved oxygen (mg/L)	7.8	7.6	7.6	7.9	7.8	7.8
	Conductivity (µmhos/cm)	2820		2820		2880	
	Temperature (°C)	25.0	24.9	24.8	24.9	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 274**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	N	BSL	BSL	N	N	EC N	EC N	EC
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.63	7.55	7.09	7.63	7.70	7.35	7.50	7.82
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.6	7.6	7.8	7.7	7.9
	Conductivity (µmhos/cm)	297		293		305		300	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)		08-09-22	88				08-09-22	
	Temperature (°C)	24.8	25.1	24.8	25.1	24.8	25.1	24.9	25.0
600 mg NaCl/L	pH (S.U.)	7.75	7.62	7.43	7.64	7.02	7.52	7.64	7.84
	Dissolved oxygen (mg/L)	7.8	7.8	7.7	7.6	7.6	7.8	7.7	7.9
	Conductivity (µmhos/cm)	1380		1360		1400		1380	
	Temperature (°C)	25.0	24.9	24.8	25.0	24.9	24.9	24.9	24.8
800 mg NaCl/L	pH (S.U.)	7.75	7.61	7.49	7.70	7.02	7.56	7.64	7.84
	Dissolved oxygen (mg/L)	7.8	7.8	7.7	7.7	7.7	7.8	7.8	7.8
	Conductivity (µmhos/cm)	1710		1690		1770		1750	
	Temperature (°C)	24.8	24.9	24.9	25.2	24.9	24.9	25.0	25.0
1000 mg NaCl/L	pH (S.U.)	7.76	7.59	7.49	7.69	7.02	7.58	7.65	7.83
	Dissolved oxygen (mg/L)	7.9	7.8	7.8	7.7	7.7	7.8	7.8	7.9
	Conductivity (µmhos/cm)	2070		2030		2120	08-08-22 EC 2110 ~~217~~		
	Temperature (°C)	24.8	25.2	24.9	25.2	24.9	25.2	24.9	25.1
1200 mg NaCl/L	pH (S.U.)	7.75	7.56	7.57	7.69	7.03	7.61	7.67	7.82
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.7	7.7	7.9	7.8	7.9
	Conductivity (µmhos/cm)	2450		2390		2490		2490	
	Temperature (°C)	24.8	24.8	25.0	25.2	25.0	25.0	25.0	25.1
1400 mg NaCl/L	pH (S.U.)	7.77	7.51	7.57	7.71	7.05	7.63	7.68	7.82
	Dissolved oxygen (mg/L)	7.9	8.0	8.0	7.7	7.8	7.9	7.8	7.9
	Conductivity (µmhos/cm)	2820		2770		2840		2840	
	Temperature (°C)	24.9	24.8	25.0	25.0	25.0	25.2	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} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic $IC_{25} \pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	04-06-21	1.1074	0.0387	0.0363	0.0041	1.0872	1.0666	1.1082	1.0683	1.1061	1.0002	1.1742
2	05-04-21	1.1043	0.0443	0.0372	0.0037	1.0895	1.0710	1.1083	1.0725	1.1065	1.0024	1.1767
3	06-08-21	1.1092	0.0431	0.0376	0.0039	1.0906	1.0712	1.1103	1.0728	1.1083	1.0033	1.1778
4	07-14-21	1.0919	0.0450	0.0381	0.0042	1.0917	1.0707	1.1130	1.0725	1.1108	1.0043	1.1790
5	08-03-21	1.0964	0.0382	0.0382	0.0042	1.0919	1.0710	1.1131	1.0728	1.1110	1.0045	1.1792
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

Warning Limits = Mean logarithmic IC_{25} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

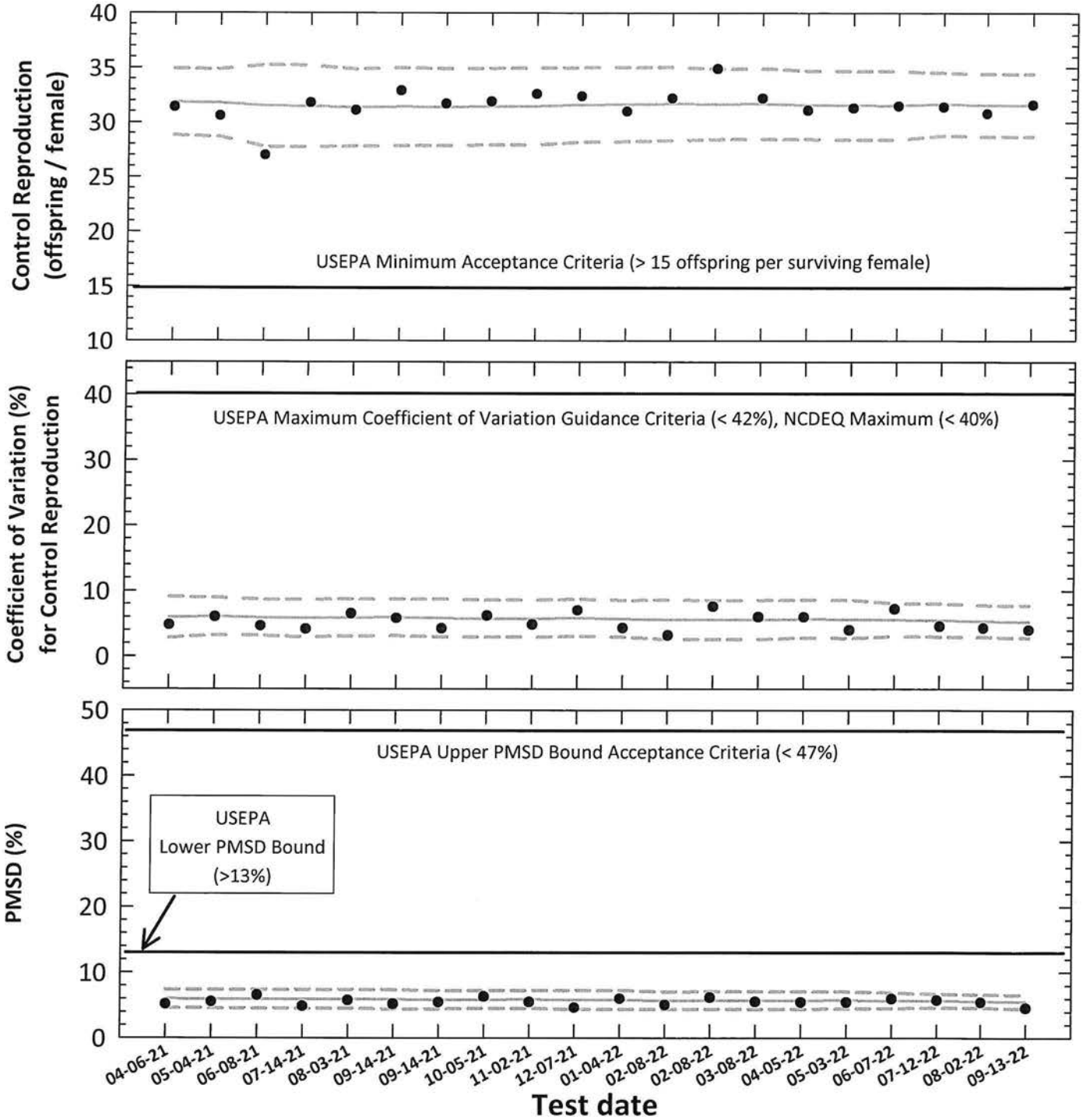
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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)

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ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control Survival	Control Reproduction		Test										
			Mean	CV	MSD	PMSD	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
		(%)	(offspring/female)	(%)		(%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	04-06-21	100	31.4	4.8	1.615	5.1	31.9	28.8	34.9	5.9	2.8	9.1	6.0	4.6	7.4
2	05-04-21	100	30.6	6.0	1.690	5.5	31.8	28.7	34.9	6.1	3.2	8.9	5.9	4.5	7.3
3	06-08-21	100	27.0	4.6	1.762	6.5	31.5	27.8	35.2	5.9	3.1	8.7	5.9	4.5	7.4
4	07-14-21	100	31.8	4.1	1.546	4.9	31.5	27.8	35.2	5.8	2.9	8.6	5.9	4.5	7.4
5	08-03-21	100	31.1	6.5	1.785	5.7	31.3	27.8	34.8	5.9	3.1	8.7	5.9	4.5	7.4
6	09-14-21	100	32.9	5.8	1.698	5.2	31.4	27.9	35.0	5.9	3.1	8.7	5.9	4.4	7.3
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	04-05-22	100	31.1	6.0	1.691	5.4	31.6	28.5	34.7	5.7	2.8	8.6	5.7	4.4	7.0
16	05-03-22	100	31.3	4.0	1.707	5.5	31.6	28.4	34.7	5.7	2.7	8.6	5.7	4.4	7.0
17	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
18	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
19	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
20	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

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.

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 275

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2130				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	09-13-22 0513 TO 0745									
Culture board:	09-06-22 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	2	3	8	9	10	18	23	24	30	31
Transfer vessel information:	pH (S.U.): 7.82 Temperature (°C): 24.9									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

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

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	09-13-22	0800	09-01-22	09-01-22	09-08-22B	JH
1	09-14-22	0745	↓	↓	↓	JH
2	09-15-22	0744	↓	↓	09-08-22D	JH
3	09-16-22	0743	↓	↓	↓	JH
4	09-17-22	0845	↓	↓	09-14-22A	JH
5	09-18-22	0842	↓	↓	↓	JH
6	09-19-22	0743	↓	↓	09-15-22A	JH
7	09-20-22	0700				JH

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

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312562
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312562
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312562
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% of Male Adults:	0%	≤ 20%	**7-day LC_{50}** (mg/L NaCl)	>1400
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	31.6	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	4.0%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1093.1

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 275**

CONTROL ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	4	5	4	4	4	6	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	12	9	12	12	11	12	12	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	15	18	15	14	19	16	13	16	17
Total young produced		31	32	31	32	30	34	32	31	30	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd 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:	0%
Mean Offspring/Female:	31.6

600 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	5	5	5	4	4	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	10	12	13	12	12	12	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	18	17	18	14	19	15	14	19	14
Total young produced		32	35	32	35	32	35	31	31	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:	0%
Mean Offspring/Female:	32.6
% Reduction from Control:	-3.2%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 275**

800 mg NaCl/L ***Survival and Reproduction Data***

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.2
% Reduction from Control:	1.3%

1000 mg NaCl/L ***Survival and Reproduction Data***

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	29.8
% Reduction from Control:	5.7%

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 275**

1200 mg NaCl/L ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	3	4	3	3	3	5	2	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	8	9	5	7	10	6	5	8	7	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	8	4	10	8	4	4	10	9	5	8
Total young produced		19	17	18	18	17	15	17	21	16	17
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.5
% Reduction from Control:	44.6%

1400 mg NaCl/L ***Survival and Reproduction Data***

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	1.7
% Reduction from Control:	94.6%

Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	4	5	4	4	4	6	4	4	45
5	11	12	9	12	12	11	12	12	10	12	113
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	18	15	14	19	16	13	16	17	158
Total	**31**	**32**	**31**	**32**	**30**	**34**	**32**	**31**	**30**	**33**	**316**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	5	5	5	4	4	5	4	4	46
5	12	12	10	12	13	12	12	12	10	12	117
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	17	18	14	19	15	14	19	14	163
Total	**32**	**35**	**32**	**35**	**32**	**35**	**31**	**31**	**33**	**30**	**326**

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	4	5	5	4	4	3	4	4	42
5	12	11	12	10	12	11	10	13	10	10	111
6	0	0	0	0	0	0	0	0	0	0	0
7	16	15	16	16	14	16	18	15	17	16	159
Total	**32**	**31**	**32**	**31**	**31**	**31**	**32**	**31**	**31**	**30**	**312**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	4	4	4	5	4	6	4	3	44
5	10	10	10	10	12	11	12	10	10	12	107
6	0	0	0	0	0	0	0	0	0	0	0
7	13	16	15	15	12	16	15	11	17	17	147
Total	**28**	**31**	**29**	**29**	**28**	**32**	**31**	**27**	**31**	**32**	**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	3	3	3	5	2	4	4	4	35
5	8	9	5	7	10	6	5	8	7	5	70
6	0	0	0	0	0	0	0	0	0	0	0
7	8	4	10	8	4	4	10	9	5	8	70
Total	**19**	**17**	**18**	**18**	**17**	**15**	**17**	**21**	**16**	**17**	**175**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	2	0	2	1	3	1	2	2	16
5	0	0	0	1	0	0	0	0	0	0	1
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	**2**	**1**	**2**	**1**	**2**	**1**	**3**	**1**	**2**	**2**	**17**

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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 #275

Test dates: September 13-20, 2022

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	31	32	31	32	30	34	32	31	30	33	100	31.6	4.0	Not applicable
600	32	35	32	35	32	35	31	31	33	30	100	32.6	5.6	-3.2
800	32	31	32	31	31	31	32	31	31	30	100	31.2	2.0	1.3
1000	28	31	29	29	28	32	31	27	31	32	100	29.8	6.1	5.7
1200	19	17	18	18	17	15	17	21	16	17	100	17.5	9.4	44.6
1400	2	1	2	1	2	1	3	1	2	2	100	1.7	39.7	94.6

Dunnett's MSD value: 1.437

PMSD: 4.5

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	9/13/2022	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	9/20/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	31.000	32.000	31.000	32.000	30.000	34.000	32.000	31.000	30.000	33.000
600	32.000	35.000	32.000	35.000	32.000	35.000	31.000	31.000	33.000	30.000
800	32.000	31.000	32.000	31.000	31.000	31.000	32.000	31.000	31.000	30.000
1000	28.000	31.000	29.000	29.000	28.000	32.000	31.000	27.000	31.000	32.000
1200	19.000	17.000	18.000	18.000	17.000	15.000	17.000	21.000	16.000	17.000
1400	2.000	1.000	2.000	1.000	2.000	1.000	3.000	1.000	2.000	2.000

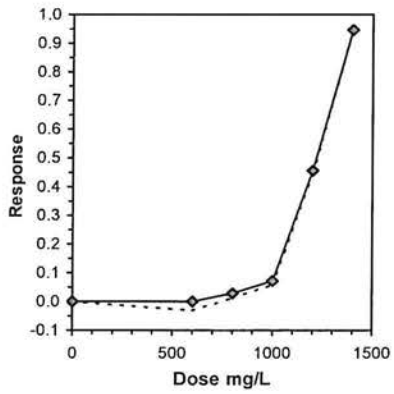
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	31.600	1.0000	31.600	30.000	34.000	4.003	10			32.100	1.0000
600	32.600	1.0316	32.600	30.000	35.000	5.638	10	121.00	75.00	32.100	1.0000
800	31.200	0.9873	31.200	30.000	32.000	2.027	10	96.50	75.00	31.200	0.9720
1000	29.800	0.9430	29.800	27.000	32.000	6.086	10	78.50	75.00	29.800	0.9283
*1200	17.500	0.5538	17.500	15.000	21.000	9.428	10	55.00	75.00	17.500	0.5452
*1400	1.700	0.0538	1.700	1.000	3.000	39.703	10	55.00	75.00	1.700	0.0530

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.85629	1.035	0.30756	0.0169
Bartlett's Test indicates unequal variances (p = 5.73E-03)	16.4254	15.0863		

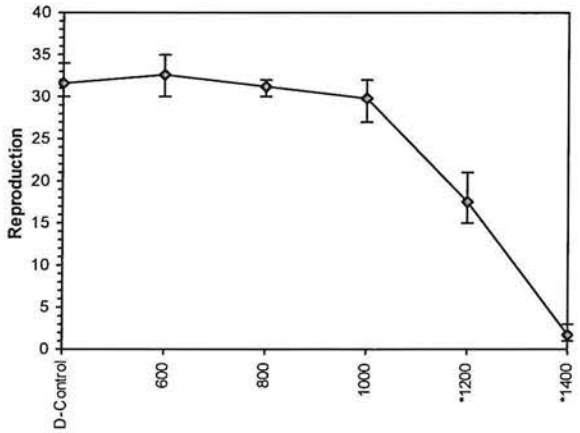
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1000	1200	1095.45	
Treatments vs D-Control				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	900.714	59.893	787.7	1007.38	0.1498
IC10	1014.8	14.626	970.574	1031.78	-2.1660
IC15	1040.89	8.42772	1022.46	1055.45	-0.1545
IC20	1066.99	7.47689	1050.49	1079.37	-0.0745
IC25	1093.09	6.7814	1078.84	1104.59	0.0268
IC40	1171.38	6.8903	1158.11	1185.37	0.3566
IC50	1218.35	5.42458	1207.22	1228.83	-0.0536



Dose-Response Plot



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Environmental Testing Solutions, Inc.

Statistical Analyses

Used for PMSD calculation only.

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	9/13/2022	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	9/20/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

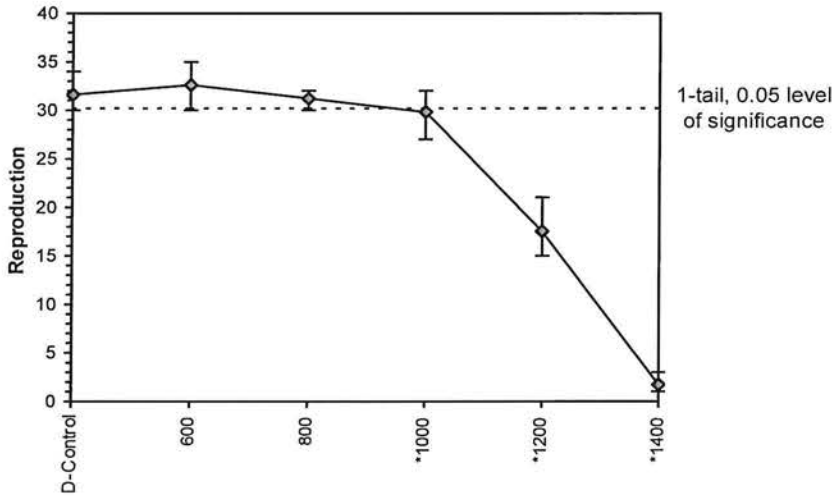
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	31.000	32.000	31.000	32.000	30.000	34.000	32.000	31.000	30.000	33.000
600	32.000	35.000	32.000	35.000	32.000	35.000	31.000	31.000	33.000	30.000
800	32.000	31.000	32.000	31.000	31.000	31.000	32.000	31.000	31.000	30.000
1000	28.000	31.000	29.000	29.000	28.000	32.000	31.000	27.000	31.000	32.000
1200	19.000	17.000	18.000	18.000	17.000	15.000	17.000	21.000	16.000	17.000
1400	2.000	1.000	2.000	1.000	2.000	1.000	3.000	1.000	2.000	2.000

			Transform: Untransformed						1-Tailed	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	31.600	1.0000	31.600	30.000	34.000	4.003	10			
600	32.600	1.0316	32.600	30.000	35.000	5.638	10	-1.591	2.287	1.437
800	31.200	0.9873	31.200	30.000	32.000	2.027	10	0.637	2.287	1.437
*1000	29.800	0.9430	29.800	27.000	32.000	6.086	10	2.865	2.287	1.437
*1200	17.500	0.5538	17.500	15.000	21.000	9.428	10	22.440	2.287	1.437
*1400	1.700	0.0538	1.700	1.000	3.000	39.703	10	47.585	2.287	1.437

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.85629	1.035	0.30756	0.0169
Bartlett's Test indicates unequal variances (p = 5.73E-03)	16.4254	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		1.43681	0.04547	1513.43	1.97407	0.0E+00	5, 54
Treatments vs D-Control										

Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 275

Daily Chemistry:

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

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	EC	EC N	EC N	EC N	EC N	EC N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.20	7.40	7.22	7.36	7.15	7.47
	Dissolved oxygen (mg/L)	7.9	7.6	7.7	7.8	7.8	7.9
	Conductivity (µmhos/cm)	309		323		317	
	Alkalinity (mg $CaCO_3$/L)	61				61	
	Hardness (mg $CaCO_3$/L)	87		09.18.22		85	
	Temperature (°C)	24.9	25.2	24.8	25.0	25.0	25.1
600 mg NaCl/L	pH (S.U.)	7.55	7.41	7.41	7.47	7.46	7.54
	Dissolved oxygen (mg/L)	~~7.4~~ 7.9	7.6	7.6	7.8	7.8	7.9
	Conductivity (µmhos/cm)	1400		1440		1460	
	Temperature (°C)	25.0	25.0	24.8	25.2	25.0	24.8
800 mg NaCl/L	pH (S.U.)	7.58	7.46	7.47	7.51	7.50	7.54
	Dissolved oxygen (mg/L)	7.9	7.6	7.6	7.8	7.8	7.9
	Conductivity (µmhos/cm)	1760		1810		1780	
	Temperature (°C)	25.0	25.0	24.9	25.2	25.0	24.8
1000 mg NaCl/L	pH (S.U.)	7.61	7.50	7.50	7.55	7.52	7.55
	Dissolved oxygen (mg/L)	~~8.1~~ 7.9	7.6	7.7	7.8	7.8	7.9
	Conductivity (µmhos/cm)	2110		2170		2140	
	Temperature (°C)	25.0	25.2	24.8	24.9	25.0	24.8
1200 mg NaCl/L	pH (S.U.)	7.63	7.54	7.54	7.55	7.54	7.55
	Dissolved oxygen (mg/L)	~~8.2~~ 7.9	7.6	7.7	7.9	7.8	8.0
	Conductivity (µmhos/cm)	2460		2530		2500	
	Temperature (°C)	25.0	24.9	24.8	24.9	25.0	24.9
1400 mg NaCl/L	pH (S.U.)	7.66	7.57	7.58	7.55	7.56	7.57
	Dissolved oxygen (mg/L)	~~8.3~~ 7.9	7.6	7.7	7.9	7.9	8.0
	Conductivity (µmhos/cm)	2780		2870		2870	
	Temperature (°C)	25.0	24.9	24.8	24.9	25.1	24.9
		Initial	Final	Initial	Final	Initial	Final

* REANALYZED + CONFIRMED D.O.

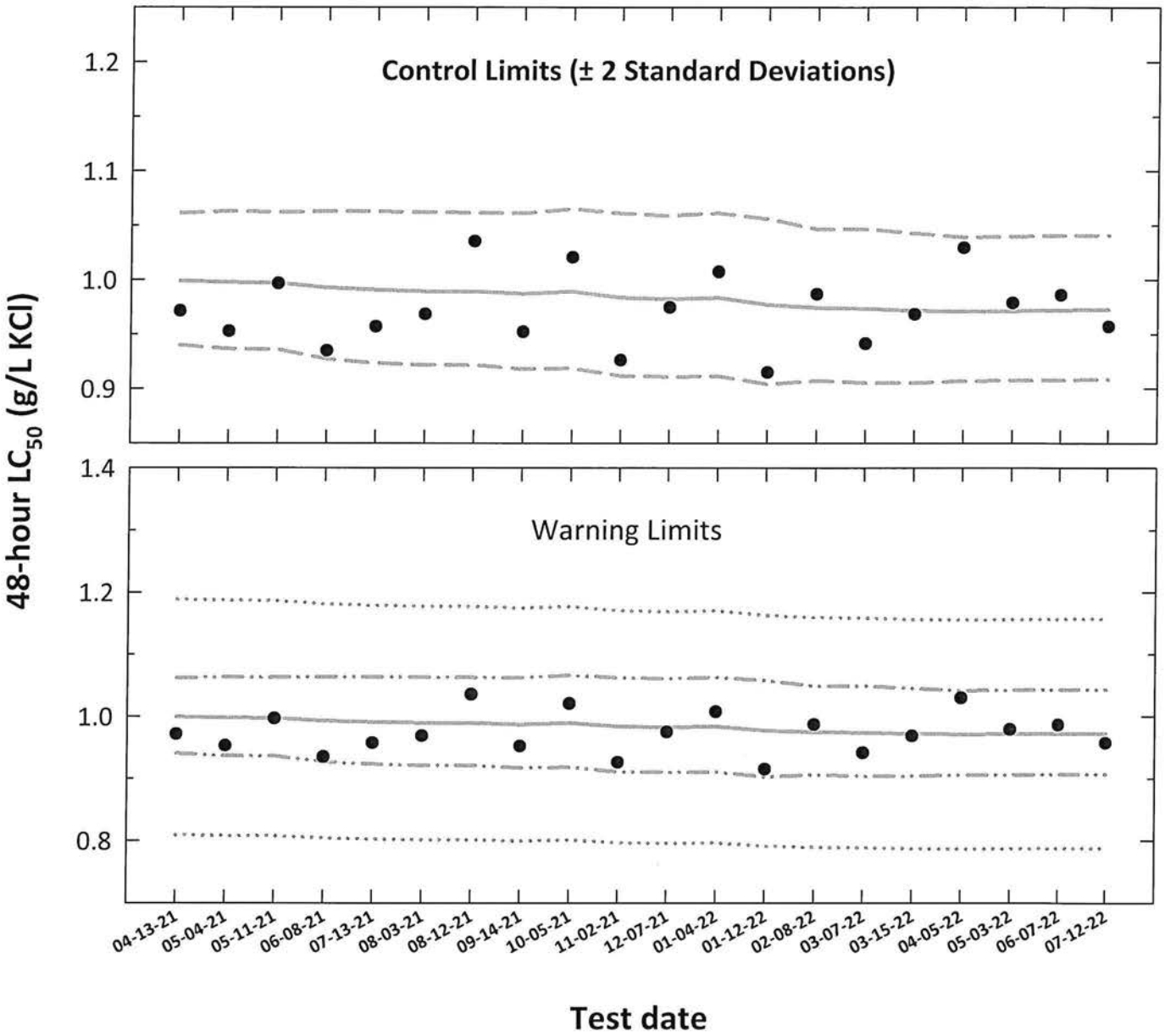
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 275

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	EC NL	BSL	BSL	BSL	BSL	EC	EC	EC
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.19	7.39	7.28	7.56	7.43	7.52	7.35	7.40
	Dissolved oxygen (mg/L)	7.7	8.0	7.7	8.0	7.7	7.9	7.8	7.9
	Conductivity (µmhos/cm)	302		310		296		313	
	Alkalinity (mg $CaCO_3$/L)								
	Hardness (mg $CaCO_3$/L)	✱ 09.18.22		87		✱ 09.18.22		87	
	Temperature (°C)	24.8	24.9	24.8	25.1	24.8	25.1	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.48	7.55	7.66	7.66	7.79	7.65	7.57	7.52
	Dissolved oxygen (mg/L)	7.7	7.9	7.8	8.0	7.8	7.8	7.8	8.0
	Conductivity (µmhos/cm)	1360		1380		1400		1440	
	Temperature (°C)	24.8	25.1	24.9	24.9	24.9	24.8	24.9	25.2
800 mg NaCl/L	pH (S.U.)	7.54	7.55	7.66	7.66	7.79	7.66	7.63	7.55
	Dissolved oxygen (mg/L)	7.7	7.9	7.8	8.0	7.8	7.8	7.9	8.0
	Conductivity (µmhos/cm)	1690		1700		1770		1840	
	Temperature (°C)	25.0	25.0	24.8	24.9	24.9	25.2	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.57	7.59	7.66	7.66	7.78	7.67	7.66	7.57
	Dissolved oxygen (mg/L)	7.7	8.0	7.8	7.9	7.9	7.9	7.9	8.0
	Conductivity (µmhos/cm)	2060		2080		2150 ~~2450~~ ✱		2230	
	Temperature (°C)	24.9	24.8	24.8	25.1	25.0	25.2	25.0	25.3
1200 mg NaCl/L	pH (S.U.)	7.57	7.60	7.64	7.68	7.77	7.66	7.68	7.57
	Dissolved oxygen (mg/L)	7.7	8.0	7.9	7.9	7.9	7.9	7.9	8.0
	Conductivity (µmhos/cm)	2360		2440		2450 ~~2500~~ ✱		2560	
	Temperature (°C)	24.9	24.9	24.8	25.1	24.9	25.0	25.0	25.0
1400 mg NaCl/L	pH (S.U.) 09.18.22	~~7.58~~ 7.62	7.61	7.63	7.69	7.73	7.66	7.70	7.58
	Dissolved oxygen (mg/L)	7.7	8.0	7.9	7.8	7.9	7.9	7.9	8.0
	Conductivity (µmhos/cm)	2710		2770		2820		2990	
	Temperature (°C)	24.9	24.9	24.8	25.0	24.9	25.0	25.0	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

✱ 09.18.22 / BSL work in wrong spot.

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	04-13-21	0.9715	-0.0126	-0.0005	0.0132	0.9989	0.9401	1.0614	0.9400	1.0615	0.8091	1.1887
2	05-04-21	0.9529	-0.0210	-0.0009	0.0138	0.9978	0.9366	1.0630	0.9365	1.0632	0.8082	1.1874
3	05-11-21	0.9967	-0.0014	-0.0012	0.0137	0.9973	0.9362	1.0623	0.9361	1.0624	0.8078	1.1867
4	06-08-21	0.9350	-0.0292	-0.0031	0.0148	0.9929	0.9273	1.0631	0.9269	1.0636	0.8042	1.1815
5	07-13-21	0.9572	-0.0190	-0.0040	0.0152	0.9909	0.9237	1.0629	0.9231	1.0636	0.8026	1.1791
6	08-03-21	0.9685	-0.0139	-0.0046	0.0154	0.9894	0.9217	1.0621	0.9210	1.0629	0.8014	1.1774
7	08-12-21	1.0354	0.0151	-0.0047	0.0153	0.9893	0.9219	1.0617	0.9212	1.0625	0.8014	1.1773
8	09-14-21	0.9522	-0.0213	-0.0057	0.0157	0.9871	0.9180	1.0613	0.9171	1.0623	0.7995	1.1746
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 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.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 138

Dilution Preparation:

Test concentrations (mg/L KCl)	500	750	1000	1250	1500
mL Stock solution	5.0	7.5	10.0	12.5	15.0
mL Dilution water	495.0	492.5	490.0	487.5	485.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2101

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	VL	TG	VL
Control, MHSW	pH (S.U.)	7.39	7.60	7.67
	Dissolved oxygen (mg/L)	7.6	7.9	7.7
	Conductivity (µmhos/cm)	293		
	Alkalinity (mg/L $CaCO_3$)	59		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.9	25.0	24.8
500 mg/L	pH (S.U.)	7.76	7.68	7.82
	Dissolved oxygen (mg/L)	7.8	8.2	7.7
	Conductivity (µmhos/cm)	1130		
	Temperature (°C)	25.0	24.8	24.9
750 mg/L	pH (S.U.)	7.75	7.68	7.83
	Dissolved oxygen (mg/L)	7.8	8.2	7.8
	Conductivity (µmhos/cm)	1520		
	Temperature (°C)	24.8	24.8	24.9
1000 mg/L	pH (S.U.)	7.76	7.71	7.86
	Dissolved oxygen (mg/L)	7.9	7.9	7.9
	Conductivity (µmhos/cm)	1920		
	Temperature (°C)	24.8	24.9	24.6
1250 mg/L	pH (S.U.)	7.78	7.71	7.86
	Dissolved oxygen (mg/L)	8.0	7.9	7.9
	Conductivity (µmhos/cm)	2350		
	Temperature (°C)	25.0	25.0	24.9
1500 mg/L	pH (S.U.)	7.80	7.74	
	Dissolved oxygen (mg/L)	8.0	8.1	
	Conductivity (µmhos/cm)	2750		
	Temperature (°C)	25.0	24.9	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130664685

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 138

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	07-12-22	*0505	JL	0705	JL	6F	LIGHT BLUE	07-07-22A
24	07-13-22			0700	JL			
48 Termination	07-14-22			0702	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:	06-30-22
Age (1 to 14 days old):	5 TO 6 DAYS
Hatch date and times:	07-06-22 1105 TO 07-07-22 0615
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.04
	Temperature (°C): 24.5

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	7 3d	6 4d	1 9d	2 8d	0 10d	0 10d
48 Termination	10	10	10	10	9 1d	9 1d	5 2d	4 2d	1	0 2d	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

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	7/12/2022	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/14/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

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

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

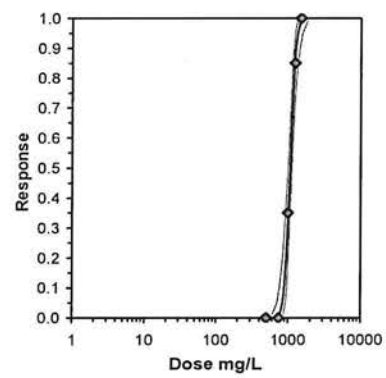
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.07271	0.07457	0.40874	0.00312	3.0E-05	4, 5
Treatments vs D-Control										

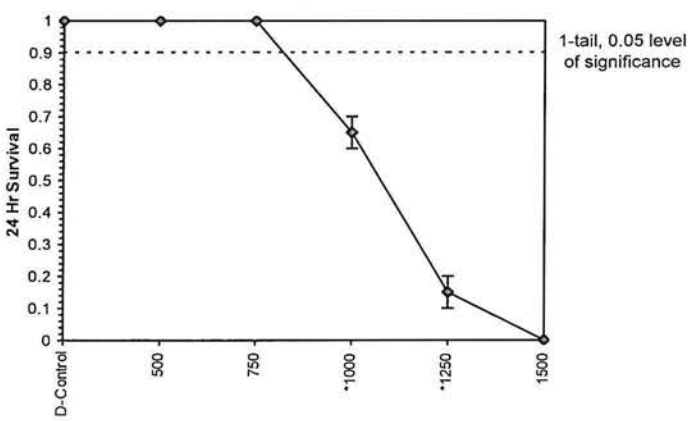
Maximum Likelihood-Probit

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

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



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	7/12/2022	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	7/14/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

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

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1726	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.690	2.850	0.1726	2	20
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	11.175	2.850	0.1726	11	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19.343	2.850	0.1726	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

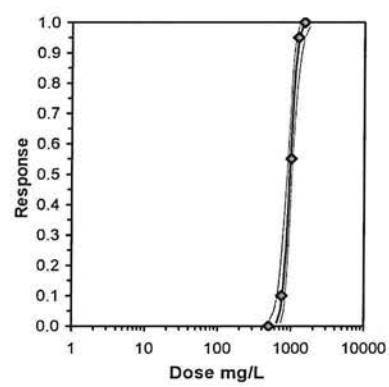
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08088	0.08295	0.52423	0.00367	2.4E-05	4, 5
Treatments vs D-Control										

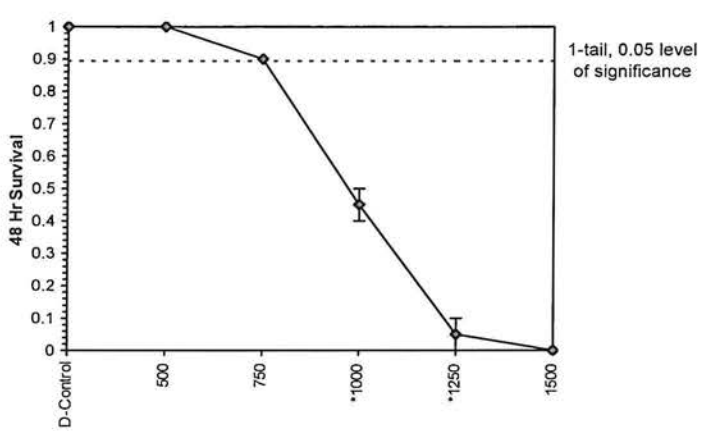
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.2642	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							
TSCR											

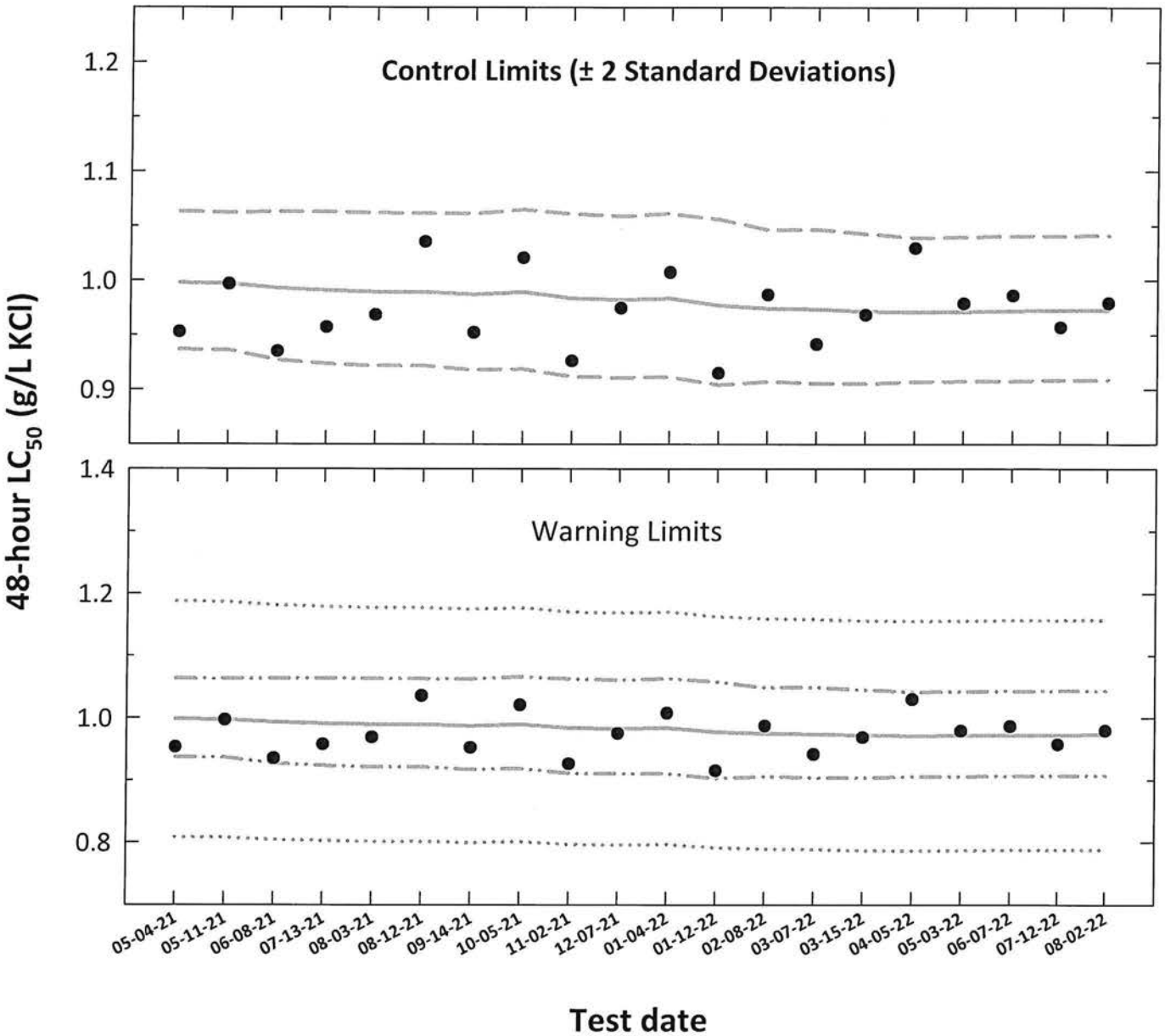
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	639.176	493.334	727.802
EC05	3.355	719.447	590.487	797.807
EC10	3.718	766.282	648.953	839.028
EC15	3.964	799.591	690.984	868.855
EC20	4.158	827.094	725.754	894.014
EC25	4.326	851.442	756.417	916.854
EC40	4.747	916.021	835.854	981.348
EC50	5.000	957.206	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 LC_{50}** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- — — — **Control Limits** (mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic LC_{50} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.75}$ converted to anti-logarithmic values, $S_{A.75}$ = 75th percentile of CVs reported nationally by USEPA)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	05-04-21	0.9529	-0.0210	-0.0009	0.0138	0.9978	0.9366	1.0630	0.9365	1.0632	0.8082	1.1874
2	05-11-21	0.9967	-0.0014	-0.0012	0.0137	0.9973	0.9362	1.0623	0.9361	1.0624	0.8078	1.1867
3	06-08-21	0.9350	-0.0292	-0.0031	0.0148	0.9929	0.9273	1.0631	0.9269	1.0636	0.8042	1.1815
4	07-13-21	0.9572	-0.0190	-0.0040	0.0152	0.9909	0.9237	1.0629	0.9231	1.0636	0.8026	1.1791
5	08-03-21	0.9685	-0.0139	-0.0046	0.0154	0.9894	0.9217	1.0621	0.9210	1.0629	0.8014	1.1774
6	08-12-21	1.0354	0.0151	-0.0047	0.0153	0.9893	0.9219	1.0617	0.9212	1.0625	0.8014	1.1773
7	09-14-21	0.9522	-0.0213	-0.0057	0.0157	0.9871	0.9180	1.0613	0.9171	1.0623	0.7995	1.1746
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 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.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 139

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 #: 2118

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	SC 08.02.22	VL	SC
Control, MHSW	pH (S.U.)	SC 7.06	7.63	7.73
	Dissolved oxygen (mg/L)	7.6	8.0	7.7
	Conductivity (µmhos/cm)	300		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.8	25.0	24.7
500 mg/L	pH (S.U.)	7.77	7.62	7.73
	Dissolved oxygen (mg/L)	7.8	7.9	7.7
	Conductivity (µmhos/cm)	1170		
	Temperature (°C)	24.9	24.7	24.6
750 mg/L	pH (S.U.)	7.81	7.63	7.74
	Dissolved oxygen (mg/L)	7.9	7.8	7.7
	Conductivity (µmhos/cm)	1610		
	Temperature (°C)	24.9	24.7	24.6
1000 mg/L	pH (S.U.)	7.82	7.64	7.75
	Dissolved oxygen (mg/L)	7.9	7.8	7.7
	Conductivity (µmhos/cm)	2010		
	Temperature (°C)	25.0	24.8	24.8
1250 mg/L	pH (S.U.)	7.83	7.63	7.75
	Dissolved oxygen (mg/L)	7.9	7.8	7.8
	Conductivity (µmhos/cm)	2420		
	Temperature (°C)	25.0	24.8	24.8
1500 mg/L	pH (S.U.)	7.83	7.63	
	Dissolved oxygen (mg/L)	7.9	7.7	
	Conductivity (µmhos/cm)	2830		
	Temperature (°C)	25.0	24.8	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066465

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 139

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	08-02-22	* 0505	JL	0705	JL	6B	ORANGE	07-29-22B
24	08-03-22			0700	JL			
48 Termination	08-04-22			0700	JL			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	07-21-22
Age (1 to 14 days old):	5 TO 6 DAYS
Hatch date and times:	07-27-22 1355 TO 07-28-22 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.92
	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	10	5 5d	6 4d	2 8d	2 8d	0 10d	0 10d
48 Termination	10	10	10	10	9 1d	9 1d	4 1d	6	1 1d	1 1d	0	0
Mean Survival	100%		100%		90%		50%		10%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	902.0
Upper 95% confidence limit (mg KCl/L)	1052.7
48-hour LC_{50} (mg KCl/L)	979.4

Comments:

Test Reviewed by: JL

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	8/2/2022	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/4/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.9000
1000	0.4000	0.6000
1250	0.1000	0.1000
1500	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1815	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.559	2.850	0.1815	2	20
*1000	0.5000	0.5000	0.7854	0.6847	0.8861	18.129	2	9.841	2.850	0.1815	10	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	17.122	2.850	0.1815	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

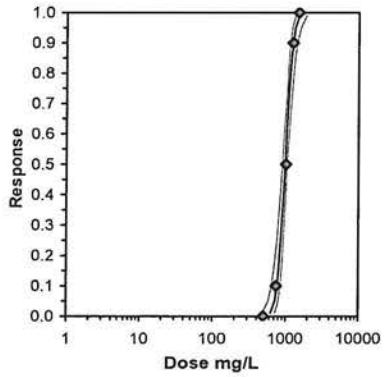
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08637	0.08859	0.45056	0.00405	4.5E-05	4, 5
Treatments vs D-Control										

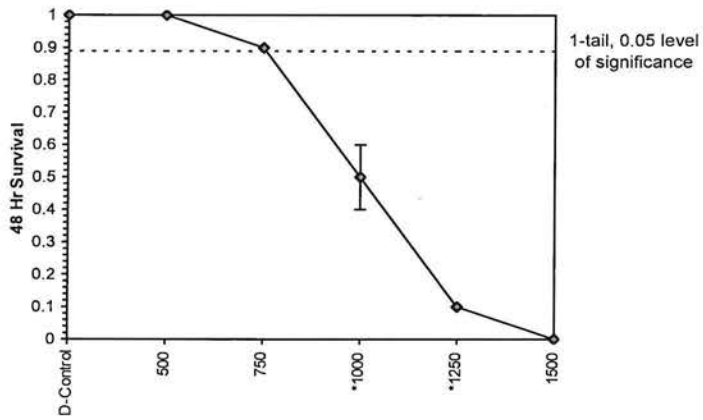
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.2001	2.17755	7.93208	16.4681	0	0.52701	7.81472	0.91292	2.99096	0.08197	3
Intercept	-31.49	6.55042	-44.329	-18.651							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	631.358	481.687	724.713
EC05	3.355	718.019	584.235	801.02
EC10	3.718	768.979	646.617	846.147
EC15	3.964	805.389	691.761	878.868
EC20	4.158	835.553	729.292	906.5
EC25	4.326	862.33	762.529	931.605
EC40	4.747	933.669	849.239	1002.59
EC50	5.000	979.398	901.956	1052.67
EC60	5.253	1027.37	953.665	1110.22
EC75	5.674	1112.36	1035.71	1225.27
EC80	5.842	1148.01	1067.06	1277.91
EC85	6.036	1191	1103.09	1344.22
EC90	6.282	1247.39	1148.09	1435.12
EC95	6.645	1335.93	1215.17	1585.22
EC99	7.326	1519.3	1345.7	1919.01



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	8/2/2022	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	8/4/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.5000	0.6000
1250	0.2000	0.2000
1500	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
*1000	0.5500	0.5500	0.8357	0.7854	0.8861	8.518	2	18.101	2.850	0.0907	9	20
*1250	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	2	29.788	2.850	0.0907	16	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

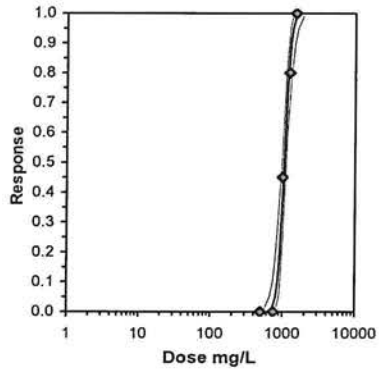
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.03598	0.0369	0.3833	0.00101	2.2E-06	4, 5
Treatments vs D-Control										

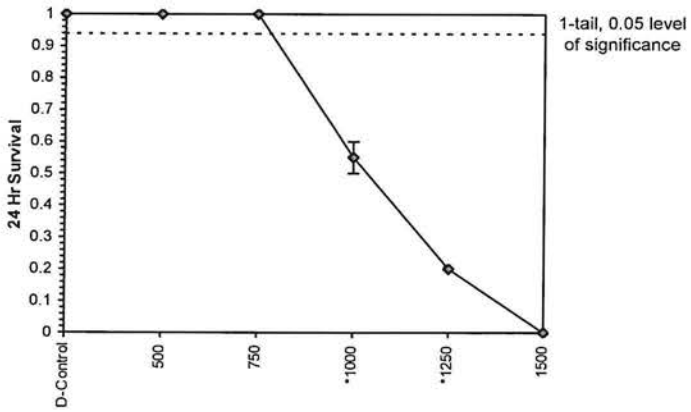
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.5196	2.73513	9.15874	19.8805	0	1.7101	7.81472	0.63469	3.02205	0.06887	5
Intercept	-38.879	8.30214	-55.151	-22.607							
TSCR											

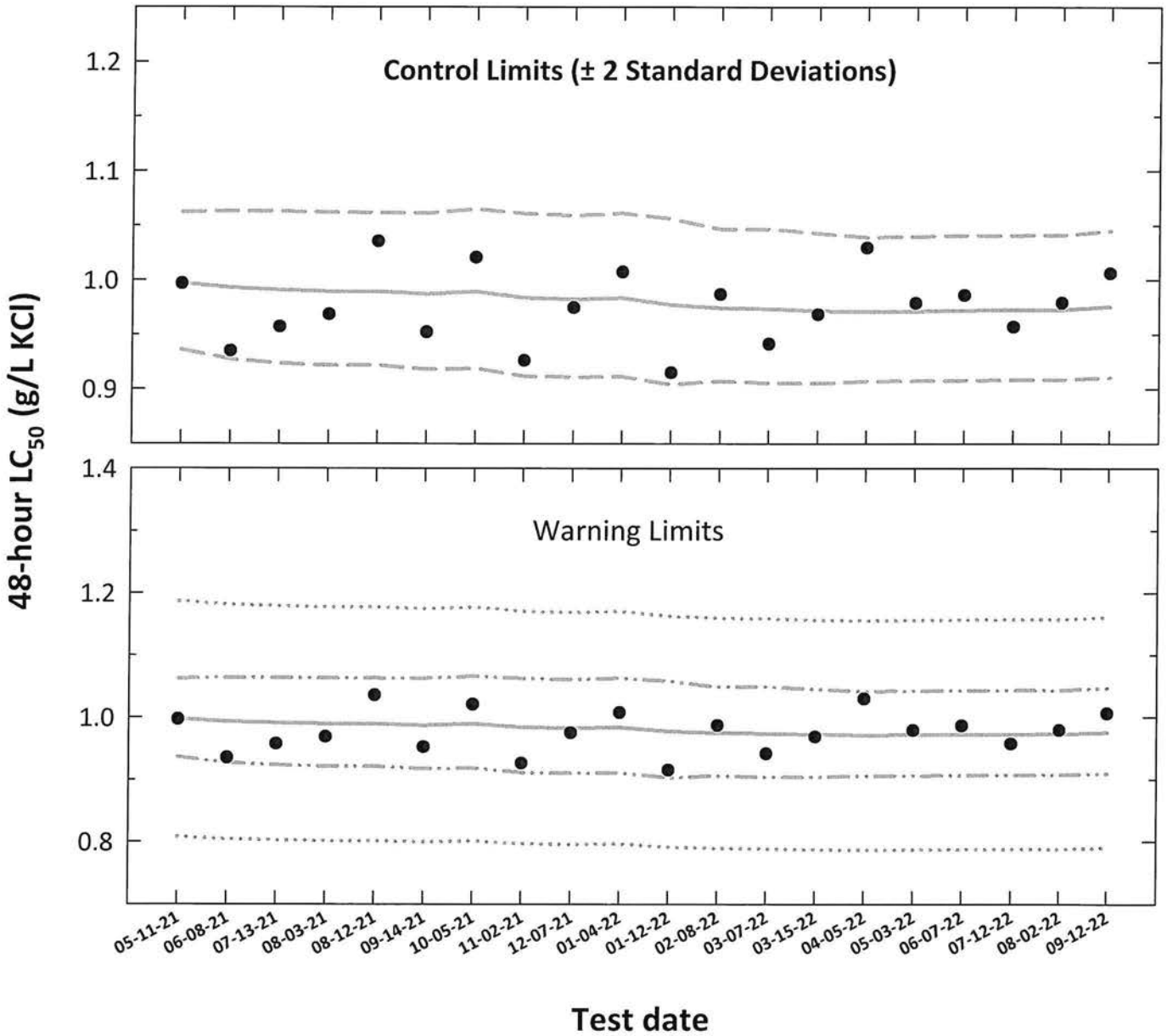
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	727.493	568.988	820.462
EC05	3.355	810.522	672.395	891.715
EC10	3.718	858.591	734.036	933.415
EC15	3.964	892.623	778.102	963.493
EC20	4.158	920.631	814.406	988.813
EC25	4.326	945.358	846.308	1011.77
EC40	4.747	1010.65	928.417	1076.55
EC50	5.000	1052.08	977.497	1122.19
EC60	5.253	1095.21	1024.97	1174.56
EC75	5.674	1170.86	1098.96	1278.71
EC80	5.842	1202.3	1126.82	1326.03
EC85	6.036	1240.03	1158.6	1385.3
EC90	6.282	1289.18	1197.97	1465.96
EC95	6.645	1365.64	1256.05	1597.73
EC99	7.326	1521.5	1367.31	1885.1



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	05-11-21	0.9967	-0.0014	-0.0012	0.0137	0.9973	0.9362	1.0623	0.9361	1.0624	0.8078	1.1867
2	06-08-21	0.9350	-0.0292	-0.0031	0.0148	0.9929	0.9273	1.0631	0.9269	1.0636	0.8042	1.1815
3	07-13-21	0.9572	-0.0190	-0.0040	0.0152	0.9909	0.9237	1.0629	0.9231	1.0636	0.8026	1.1791
4	08-03-21	0.9685	-0.0139	-0.0046	0.0154	0.9894	0.9217	1.0621	0.9210	1.0629	0.8014	1.1774
5	08-12-21	1.0354	0.0151	-0.0047	0.0153	0.9893	0.9219	1.0617	0.9212	1.0625	0.8014	1.1773
6	09-14-21	0.9522	-0.0213	-0.0057	0.0157	0.9871	0.9180	1.0613	0.9171	1.0623	0.7995	1.1746
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 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.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 140

Dilution Preparation:

Test concentrations (mg/L KCl)	500	750	1000	1250	1500
mL Stock solution	5.0	7.5	10.0	12.5	15.0
mL Dilution water	495.0	492.5	490.0	487.5	485.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2128

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	SC	SC	SC N
Control, MHSW	pH (S.U.)	7.10	7.30	7.54
	Dissolved oxygen (mg/L)	7.7	~~7.9~~ 7.6	7.8
	Conductivity (µmhos/cm)	305		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	85		
	Temperature (°C)	24.9	24.6	24.7
500 mg/L	pH (S.U.)	7.62	7.45	7.60
	Dissolved oxygen (mg/L)	7.9	7.7	7.8
	Conductivity (µmhos/cm)	1150		
	Temperature (°C)	24.7	24.8	24.7
750 mg/L	pH (S.U.)	7.66	7.47	7.59
	Dissolved oxygen (mg/L)	7.9	7.7	7.8
	Conductivity (µmhos/cm)	1580		
	Temperature (°C)	24.9	24.8	24.5
1000 mg/L	pH (S.U.)	7.68	7.48	7.61
	Dissolved oxygen (mg/L)	8.0	7.7	7.7
	Conductivity (µmhos/cm)	1990		
	Temperature (°C)	24.9	24.9	24.6
1250 mg/L	pH (S.U.)	7.70	7.51	7.63
	Dissolved oxygen (mg/L)	8.0	7.6	7.8
	Conductivity (µmhos/cm)	2400 ~~2250~~	N	
	Temperature (°C)	24.8	24.9	24.6
1500 mg/L	pH (S.U.)	7.72	7.63	7.62
	Dissolved oxygen (mg/L)	8.0	7.6	7.8
	Conductivity (µmhos/cm)	2830		
	Temperature (°C)	24.9	24.7	24.6

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	13066485

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 140

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	09-12-22	* 0705	JL	0915	JL	2F	RED	09-06-22
24	09-13-22			0916	JL			
48 Termination	09-14-22			0915	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:	08-30-22
Age (1 to 14 days old):	5 TO 6 DAYS
Hatch date and times:	09-06-22 1050 TO 09-07-22 0650
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.83
	Temperature (°C): 24.0

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	6 4d	6 4d	3 7d	4 6d	1 9d	1 9d
48 Termination	10	10	10	10	10	10	4 2d	4 2d	2 1d	1 3d	0 1d	0 1d
Mean Survival	100%		100%		100%		40%		15%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	933.5
Upper 95% confidence limit (mg KCl/L)	1074.8
48-hour LC_{50} (mg KCl/L)	1006.3

Comments:

Test Reviewed by: JL

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	9/12/2022	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/14/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.6000	0.6000
1250	0.3000	0.4000
1500	0.1000	0.1000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.0858	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.830	0.0858	0	20
*1000	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	17.338	2.830	0.0858	8	20
*1250	0.3500	0.3500	0.6322	0.5796	0.6847	11.753	2	25.708	2.830	0.0858	13	20
*1500	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	35.942	2.830	0.0858	18	20

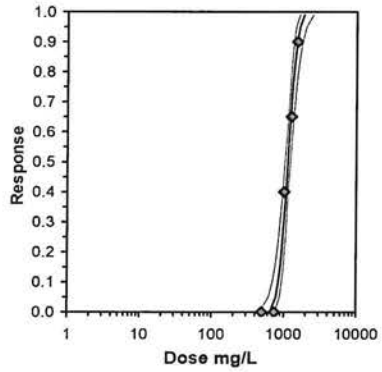
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.03366	0.03452	0.44664	0.00092	9.8E-08	5, 6
Treatments vs D-Control										

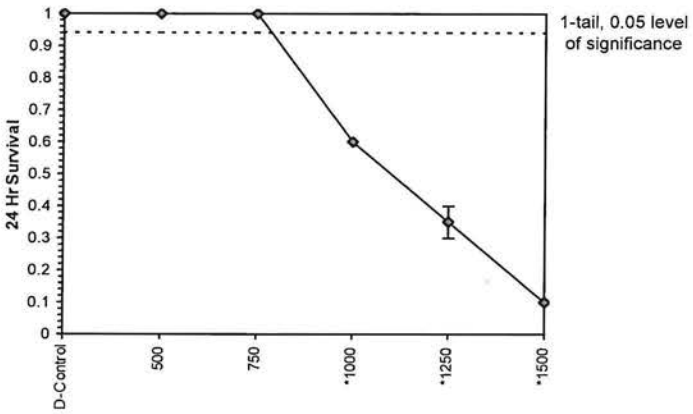
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	10.526	1.94109	6.72142	14.3305	0	1.79087	7.81472	0.61692	3.0494	0.095	5
Intercept	-27.098	5.93326	-38.727	-15.469							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	673.587	494.019	784.932
EC05	3.355	781.876	620.737	880.268
EC10	3.718	846.55	699.885	937.343
EC15	3.964	893.182	757.99	979.118
EC20	4.158	932.068	806.732	1014.72
EC25	4.326	966.775	850.145	1047.4
EC40	4.747	1060.07	963.679	1142.13
EC50	5.000	1120.48	1032.06	1211.48
EC60	5.253	1184.33	1098.17	1293.36
EC75	5.674	1298.62	1202.04	1460.56
EC80	5.842	1346.98	1241.92	1537.7
EC85	6.036	1405.62	1288.12	1635.26
EC90	6.282	1483.05	1346.48	1769.76
EC95	6.645	1605.72	1434.72	1994.12
EC99	7.326	1863.87	1609.96	2504.09



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	9/12/2022	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/14/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

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

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*1000	0.4000	0.4000	0.6847	0.6847	0.6847	0.000	2	16.208	2.850	0.1279	12	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	22.716	2.850	0.1279	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

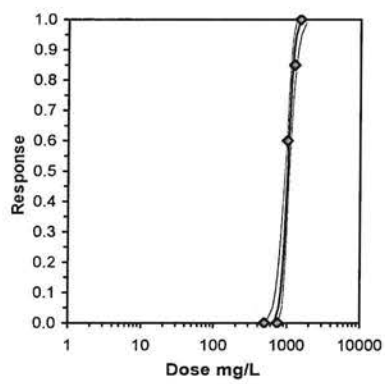
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.05495	0.05636	0.47892	0.00201	6.9E-06	4, 5
Treatments vs D-Control										

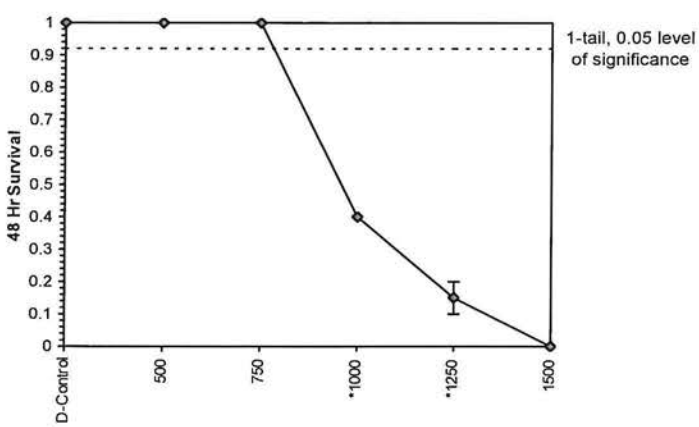
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.4862	2.71266	9.16941	19.8031	0	2.89101	7.81472	0.40874	3.00273	0.06903	6
Intercept	-38.498	8.18265	-54.536	-22.46							
TSCR											

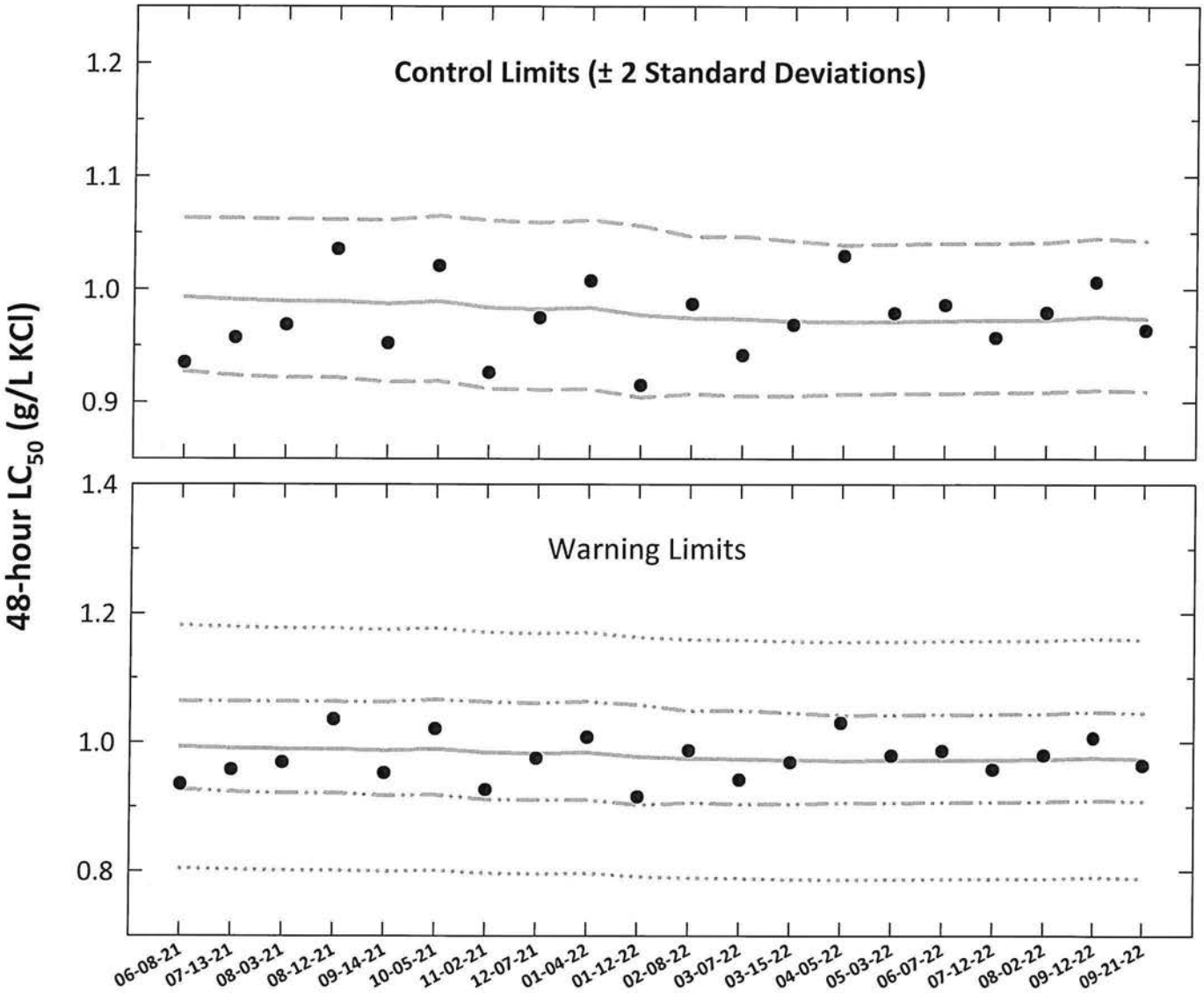
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	695.24	544.107	784.528
EC05	3.355	774.781	642.763	853.057
EC10	3.718	820.839	701.532	893.199
EC15	3.964	853.452	743.527	922.166
EC20	4.158	880.293	778.112	946.557
EC25	4.326	903.991	808.499	968.671
EC40	4.747	966.577	886.72	1031.01
EC50	5.000	1006.29	933.545	1074.83
EC60	5.253	1047.64	978.957	1124.95
EC75	5.674	1120.17	1050	1224.34
EC80	5.842	1150.33	1076.83	1269.44
EC85	6.036	1186.51	1107.45	1325.93
EC90	6.282	1233.65	1145.39	1402.81
EC95	6.645	1306.99	1201.35	1528.45
EC99	7.326	1456.52	1308.49	1802.55



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	06-08-21	0.9350	-0.0292	-0.0031	0.0148	0.9929	0.9273	1.0631	0.9269	1.0636	0.8042	1.1815
2	07-13-21	0.9572	-0.0190	-0.0040	0.0152	0.9909	0.9237	1.0629	0.9231	1.0636	0.8026	1.1791
3	08-03-21	0.9685	-0.0139	-0.0046	0.0154	0.9894	0.9217	1.0621	0.9210	1.0629	0.8014	1.1774
4	08-12-21	1.0354	0.0151	-0.0047	0.0153	0.9893	0.9219	1.0617	0.9212	1.0625	0.8014	1.1773
5	09-14-21	0.9522	-0.0213	-0.0057	0.0157	0.9871	0.9180	1.0613	0.9171	1.0623	0.7995	1.1746
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note: **48-hour LC_{50}** = 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 LC_{50} values.

S = Standard deviation of the LC_{50} values.

Control Limits = Mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values.

Warning Limits = Mean logarithmic LC_{50} ± 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.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 141

Dilution Preparation:

Test concentrations (mg/L KCl)	500	750	1000	1250	1500
mL Stock solution	5.0	7.5	10.0	12.5	15.0
mL Dilution water	495.0	492.5	490.0	487.5	485.0
Total volume (mL)	500	500	500	500	500

A stock solution was prepared by diluting 100 g KCl into 2000 mL deionized water. This 50,000 mg/L KCl stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2128

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	EC M	M	EC
Control, MHSW	pH (S.U.)	7.50	7.40	7.03
	Dissolved oxygen (mg/L)	7.6	7.3	7.5
	Conductivity (µmhos/cm)	314		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	87		
	Temperature (°C)	24.9	24.7	24.4
500 mg/L	pH (S.U.)	7.73	7.42	7.70
	Dissolved oxygen (mg/L)	7.6	7.2	7.4
	Conductivity (µmhos/cm)	1190		
	Temperature (°C)	24.8	24.6	24.6
750 mg/L	pH (S.U.)	7.75	7.46	7.70
	Dissolved oxygen (mg/L)	7.6	7.2	7.3
	Conductivity (µmhos/cm)	1610		
	Temperature (°C)	24.8	24.6	24.6
1000 mg/L	pH (S.U.)	7.75	7.49	7.70
	Dissolved oxygen (mg/L)	7.6	7.1	7.3
	Conductivity (µmhos/cm)	2050		
	Temperature (°C)	25.0	24.8	24.6
1250 mg/L	pH (S.U.)	7.76	7.45	7.68
	Dissolved oxygen (mg/L)	7.6	7.1	7.3
	Conductivity (µmhos/cm)	2460		
	Temperature (°C)	24.9	24.8	24.5
1500 mg/L	pH (S.U.)	7.76	7.43	
	Dissolved oxygen (mg/L)	7.7	7.1	
	Conductivity (µmhos/cm)	2870		
	Temperature (°C)	24.9	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	13066485

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*

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

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 141

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	09·21·22	*0520	JL	0720	JL	2F	RED	09·15·22B
24	09·22·22			0722	JL			
48 Termination	09·23·22			0715	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:	09·05·22
Age (1 to 14 days old):	8 TO 9 DAYS
Hatch date and times:	09·12·22 1455 TO 09·13·22 0500
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.71
	Temperature (°C): 24.0

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	9 1d	5 5d	5 5d	2 8d	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	10	9	3 7d	3 7d	2	1	0	0
Mean Survival	100%		100%		95%		30%		15%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	887.6
Upper 95% confidence limit (mg KCl/L)	1035.7
48-hour LC_{50} (mg KCl/L)	963.8

Comments:

Test Reviewed by: JL

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	9/21/2022	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	9/23/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	0.9000
1000	0.5000	0.5000
1250	0.2000	0.1000
1500	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1947	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.192	2.850	0.1947	1	20
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	9.170	2.850	0.1947	10	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	14.917	2.850	0.1947	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

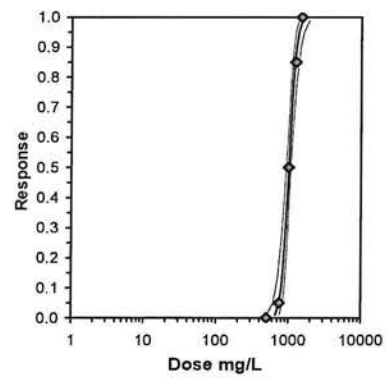
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.09486	0.09729	0.42075	0.00467	7.6E-05	4, 5
Treatments vs D-Control										

Maximum Likelihood-Probit

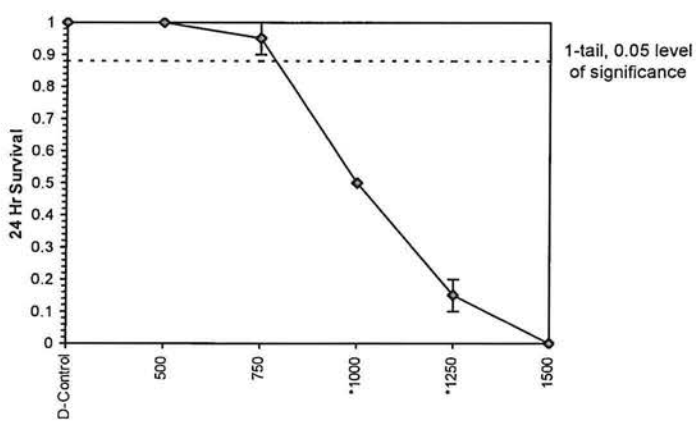
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.8027	2.30146	8.29186	17.3136	0	0.52742	7.81472	0.91283	3.00359	0.07811	3
Intercept	-33.454	6.94845	-47.073	-19.835							

TSCR

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	663.57	511.738	757.079
EC05	3.355	750.096	615.451	832.81
EC10	3.718	800.744	678.121	877.465
EC15	3.964	836.835	723.289	909.796
EC20	4.158	866.675	760.726	937.074
EC25	4.326	893.121	793.794	961.842
EC40	4.747	963.397	879.678	1031.82
EC50	5.000	1008.31	931.619	1081.12
EC60	5.253	1055.32	982.378	1137.68
EC75	5.674	1138.35	1062.61	1250.38
EC80	5.842	1173.09	1093.18	1301.76
EC85	6.036	1214.92	1128.27	1366.34
EC90	6.282	1269.68	1172.03	1454.62
EC95	6.645	1355.41	1237.1	1599.85
EC99	7.326	1532.15	1363.24	1920.72



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	9/21/2022	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	9/23/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	0.9000
1000	0.3000	0.3000
1250	0.2000	0.1000
1500	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1947	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.192	2.850	0.1947	1	20
*1000	0.3000	0.3000	0.5796	0.5796	0.5796	0.000	2	12.181	2.850	0.1947	14	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	14.917	2.850	0.1947	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

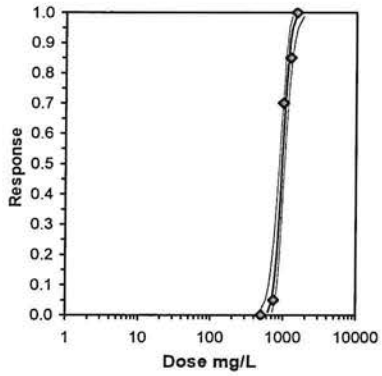
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.09486	0.09729	0.49553	0.00467	5.1E-05	4, 5
Treatments vs D-Control										

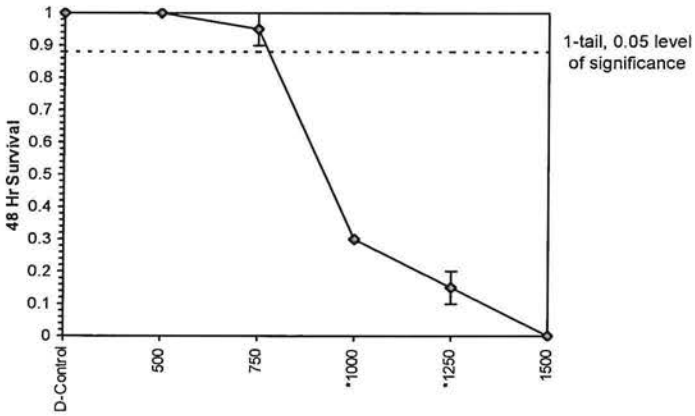
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.4383	2.23794	8.05198	16.8247	0	3.06084	7.81472	0.38234	2.98397	0.0804	3
Intercept	-32.116	6.71616	-45.279	-18.952							
TSCR											

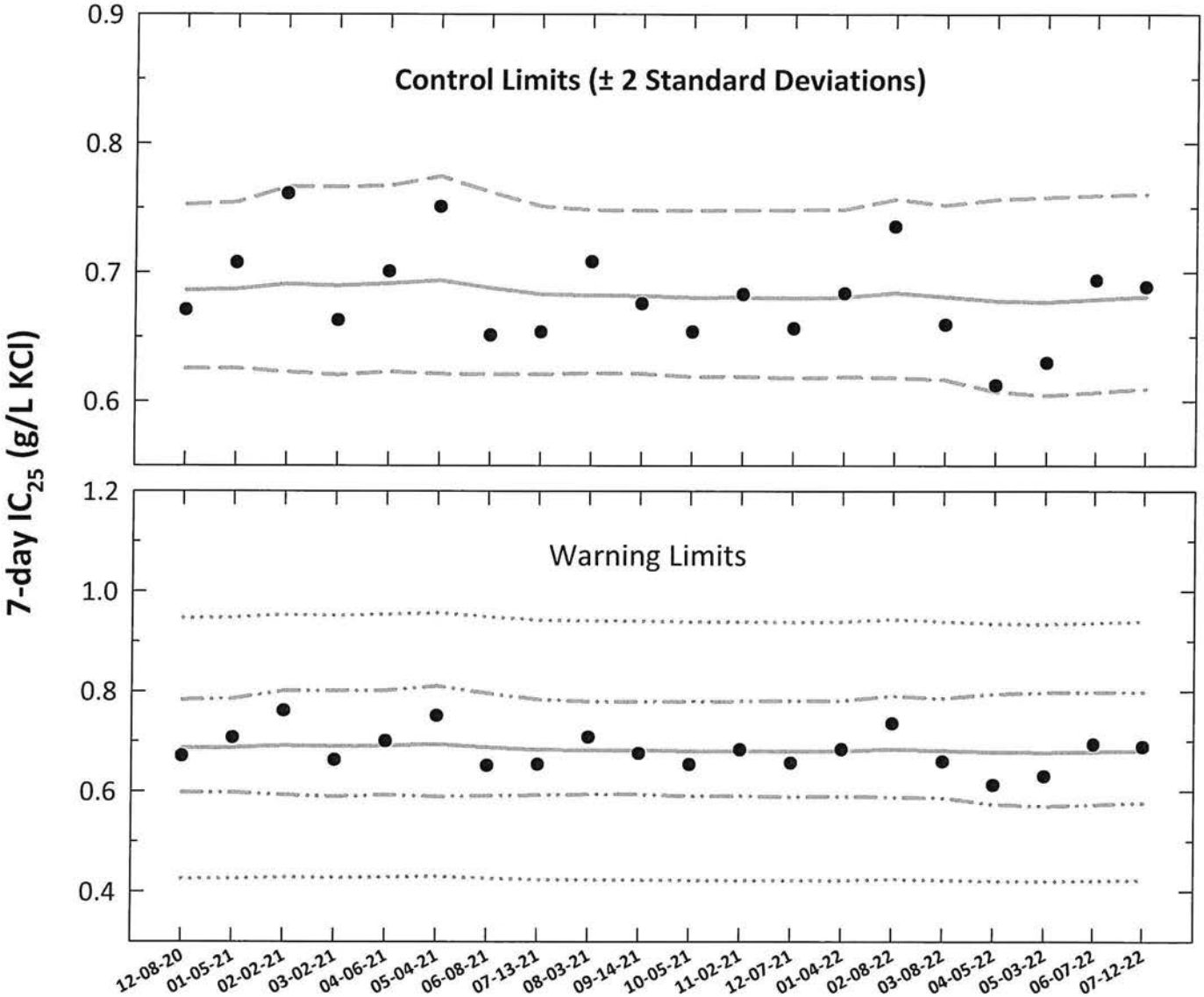
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	626.524	478.706	718.11
EC05	3.355	710.767	578.907	792.125
EC10	3.718	760.214	639.715	835.85
EC15	3.964	795.504	683.656	867.538
EC20	4.158	824.716	720.148	894.289
EC25	4.326	850.631	752.436	918.587
EC40	4.747	919.602	836.55	987.26
EC50	5.000	963.758	887.616	1035.67
EC60	5.253	1010.03	937.66	1091.24
EC75	5.674	1091.93	1016.97	1202.25
EC80	5.842	1126.24	1047.22	1253.01
EC85	6.036	1167.6	1081.96	1316.91
EC90	6.282	1221.8	1125.3	1404.46
EC95	6.645	1306.8	1189.79	1548.88
EC99	7.326	1482.51	1314.98	1869.45



Dose-Response Plot



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



Test date

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

Independent Review by Kelley E. Keenan:

Entered and Reviewed by Jim Sumner

Independent Review by Kelley E. Keenan:

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	12-08-20	0.6708	-0.1734	-0.1636	0.0201	0.6861	0.6255	0.7526	0.5977	0.7830	0.4254	0.9468
2	01-05-21	0.7076	-0.1502	-0.1630	0.0203	0.6870	0.6257	0.7544	0.5977	0.7850	0.4259	0.9481
3	02-02-21	0.7611	-0.1186	-0.1606	0.0226	0.6909	0.6228	0.7666	0.5923	0.8004	0.4284	0.9535
4	03-02-21	0.6628	-0.1786	-0.1615	0.0229	0.6895	0.6205	0.7662	0.5894	0.8008	0.4275	0.9515
5	04-06-21	0.7006	-0.1545	-0.1603	0.0226	0.6914	0.6230	0.7672	0.5925	0.8011	0.4287	0.9541
6	05-04-21	0.7509	-0.1244	-0.1589	0.0240	0.6937	0.6212	0.7746	0.5892	0.8103	0.4301	0.9573
7	06-08-21	0.6512	-0.1863	-0.1625	0.0222	0.6879	0.6210	0.7620	0.5907	0.7956	0.4265	0.9493
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

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

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

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

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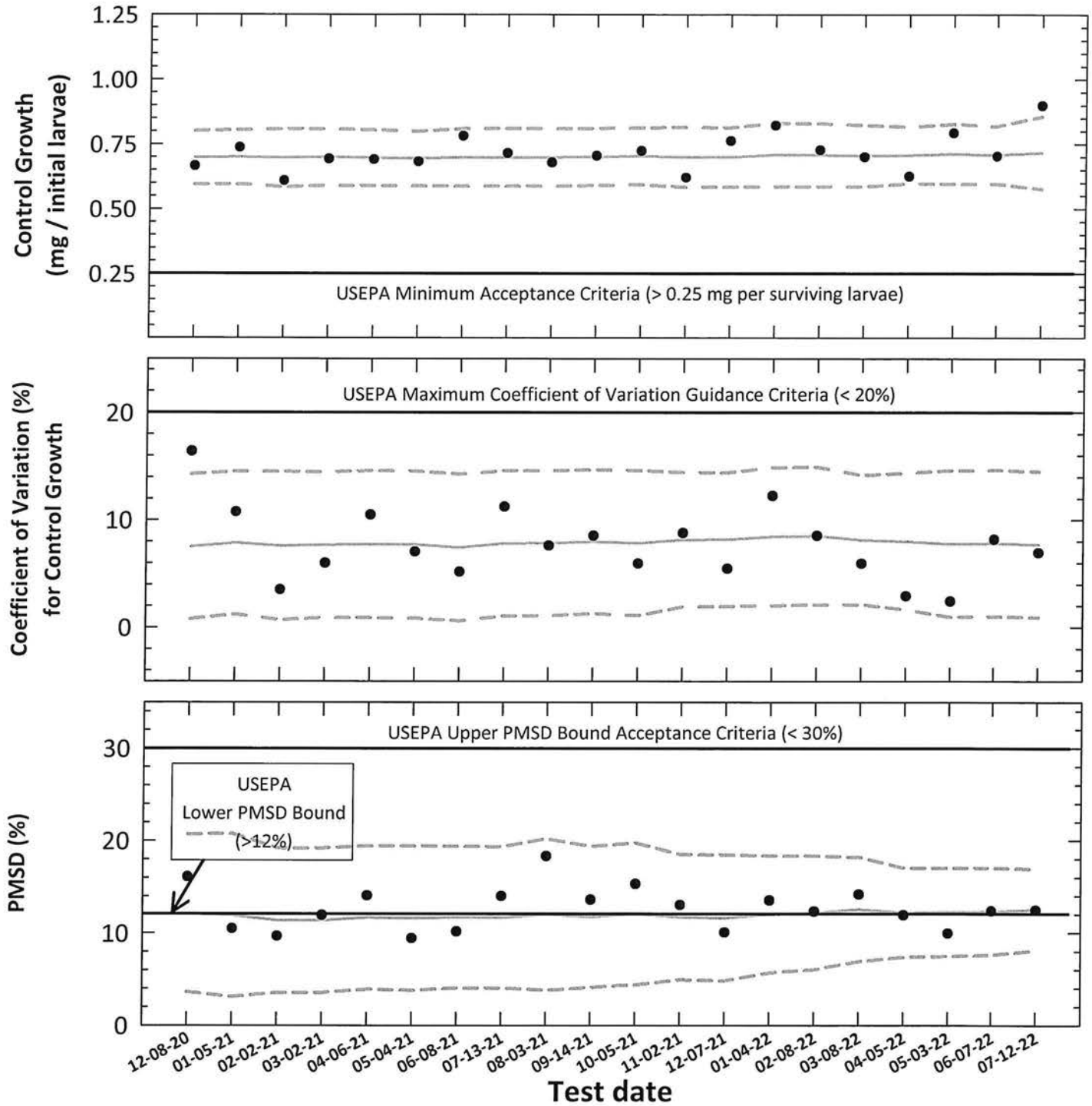
Environmental Testing Solutions, Inc.

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)

Independant Review by Kelley E. Keenan:

Entered and Reviewed by Jim Sumner

Independent Review by Kelley E. Keenan

ETS Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination: Control Survival (%)	ToxCal Determination: Control Growth Mean (mg/initial larvae)	ToxCal Determination: Control Growth CV (%)	ToxCal Determination: Test MSD	ToxCal Determination: Test PMSD (%)	Control Growth (mg/initial larvae) CT	Control Growth 95% Confidence Interval CT - 2S	Control Growth 95% Confidence Interval CT + 2S	Control Growth CV (%) CT	Control Growth CV 95% Confidence Interval CT - 2S	Control Growth CV 95% Confidence Interval CT + 2S	Test PMSD (%) CT	Test PMSD 95% Confidence Interval CT - 2S	Test PMSD 95% Confidence Interval CT + 2S
1	12-08-20	100	0.666	16.4	0.1070	16.1	0.698	0.595	0.801	7.5	0.8	14.3	12.1	3.6	20.7
2	01-05-21	100	0.737	10.8	0.0774	10.5	0.700	0.596	0.805	7.9	1.2	14.5	11.9	3.1	20.8
3	02-02-21	100	0.609	3.5	0.0588	9.7	0.697	0.585	0.809	7.6	0.7	14.5	11.4	3.5	19.2
4	03-02-21	100	0.693	6.0	0.0827	11.9	0.699	0.590	0.809	7.7	0.9	14.5	11.4	3.5	19.2
5	04-06-21	100	0.691	10.5	0.0970	14.0	0.697	0.589	0.805	7.7	0.9	14.6	11.7	3.9	19.4
6	05-04-21	100	0.682	7.1	0.0643	9.4	0.694	0.589	0.799	7.7	0.8	14.6	11.6	3.8	19.4
7	06-08-21	100	0.781	5.2	0.0794	10.2	0.699	0.587	0.811	7.4	0.6	14.3	11.7	4.0	19.4
8	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	0.810	7.8	1.1	14.6	11.7	4.0	19.3
9	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	1.1	14.6	12.0	3.8	20.2
10	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	1.3	14.7	11.7	4.1	19.4
11	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	14.6	12.1	4.4	19.7
12	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	14.4	11.7	4.9	18.5
13	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5
14	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3
15	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3
16	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2
17	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0
18	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	14.6	12.3	7.5	17.0
19	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	14.7	12.3	7.6	17.0
20	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	14.5	12.5	8.1	16.9

Note:

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

Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.

CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 12%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: 91

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2101				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	07-05-22	*Artemia* CHM number:	CHM1149
Hatch dates and times:	07-11-22 1511 TO 07-12-22 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.04 S.U. Temperature = 24.5 °C	Date / Time in oven:	07-19-22 0646
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	07-20-22 0650
		*Final oven temperature:	60 °C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	07-12-22	0505	JL	~~07-12-22~~ 1110	JL	0715	JL	07-07-22 A
1	07-13-22	0500	JL	1100	JL	0708	JL	↓
2	07-14-22	0500	JL	1100	JL	0700	JL	07-07-22 C
3	07-15-22	0500	JL	1100	JL	0700	JL	↓
4	07-16-22	0600	JL	1200	JL	0800	JL	07-12-22 A
5	07-17-22	0500	JL	1100	JL	0700	JL	↓
6	07-18-22	0500	JL	1100	JL	0700	JL	↓
7	07-19-22					0615	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	13066468S

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	848.9
Average weight per initial larvae:	0.899		**NOEC** (mg/L KCl)	450
Average weight per surviving larvae:	0.899	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	600
			ChV (mg/L KCl)	519.6
			IC_{25} (mg/L KCl)	688.7

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **91**

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: Turquoise Analyst: JR Date: 10.28.22	14.99	15.22	11.51	13.80	16.33	13.81	15.55	13.28	14.79	13.94	14.33	14.75
*B = Pan + Larvae weight (mg) Analyst: JR Date: 07-23-22	23.85	23.99	19.95	23.69	24.56	21.54	24.88	22.16	22.61	22.95	22.16	22.40
C = Larvae weight (mg) = B – A Analyst: JR	8.86	8.77	8.44	9.89	8.23	7.73	9.33	8.88	7.82	9.01	7.83	7.65
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JR	0.886	0.877	0.844	0.989	0.823	0.773	0.933	0.888	0.782	0.901	0.783	0.765
Average weight per initial number of larvae (mg)	0.899				0.854				0.808			
Percent reduction from control (%)					5.02				10.27			

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

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

Comments:

Independent Review by Kelley E. Keenan: KK

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **91**

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 d	9 d	9 d	9 d	6 d	6 d	6 d	6 d
2	10	10	10	10	8 d	9	9	9	6	6	5 d	6
3	10	10	10	10	8	9	9	9	6	6	5	6
4	10	10	10	10	8	8 d	9	8 d	4 d	5 d	5	5 d
5	10	10	10	10	8	8	8 d	7 d	4	5	5	5
6	9 d	10	10	10	8	8	8	7	3 d	5	4 d	4 d
7	9	10	10	10	8	8	8	7	3	5	4	4
*A = Pan weight (mg) Tray color code: Turquoise Analyst: JR Date: 06-28-22	15.69	15.11	14.12	13.99	14.72	JR 06-28-22 ~~14.8~~ 14.60	14.62	12.44	14.67	15.24	14.68	12.89
*B = Pan + Larvae weight (mg) Analyst: JR Date: 07-23-22	22.48	22.04	21.02	22.45	20.82	21.56	20.89	18.62	17.19	20.18	18.16	16.49
C = Larvae weight (mg) = B – A Analyst: JR	6.79	6.93	6.90	8.46	6.10	6.96	6.27	6.18	2.52	4.94	3.48	3.60
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JR	0.679	0.693	0.690	0.846	0.610	0.696	0.627	0.618	0.252	0.494	0.348	0.360
Average weight per initial number of larvae (mg)	0.727				0.638				0.364			
Percent reduction from control (%)		19.1%				29.1%				59.6%		

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

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

Comments:

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **91**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 7d	3 7d	4 6d	3 7d
2	2 1d	3	3 1d	3
3	2	2 1d	2 1d	2 1d
4	2	2	2	2
5	1 1d	1 1d	1 1d	2
6	1	1	1	2
7	0 1d	1	1	1 1d LG
*A = Pan weight (mg) Tray color code: Turquoise Analyst: JR Date: 06-28-22	15.08	13.98	12.57	12.78
*B = Pan + Larvae weight (mg) Analyst: JR Date: 07-23-22	~~07-19-22~~	14.55	13.20	13.92
C = Larvae weight (mg) = B – A Analyst: JL	~~07-19-22~~	0.57	0.63	1.14
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0	0.057	0.063	0.114
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.059		93.5%	

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

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

Comments:

Independent Review by Kelley E. Keenan: K

Independent Review by Kelley E. Keenan:

Environmental Testing Solutions, Inc.

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: 91

Test dates: July 12-19, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.99	23.85	8.86	0.886	0.899	7.0	0.886	100.0	0.899	7.0	Not applicable
	B	10	10	15.22	23.99	8.77	0.877			0.877				
	C	10	10	11.51	19.95	8.44	0.844			0.844				
	D	10	10	13.80	23.69	9.89	0.989			0.989				
300	E	10	10	16.33	24.56	8.23	0.823	0.854	8.3	0.823	100.0	0.854	8.3	5.0
	F	10	10	13.81	21.54	7.73	0.773			0.773				
	G	10	10	15.55	24.88	9.33	0.933			0.933				
	H	10	10	13.28	22.16	8.88	0.888			0.888				
450	I	10	10	14.79	22.61	7.82	0.782	0.808	7.8	0.782	100.0	0.808	7.8	10.2
	J	10	10	13.94	22.95	9.01	0.901			0.901				
	K	10	10	14.33	22.16	7.83	0.783			0.783				
	L	10	10	14.75	22.40	7.65	0.765			0.765				
600	M	10	9	15.69	22.48	6.79	0.754	0.746	9.8	0.679	97.5	0.727	10.9	19.1
	N	10	10	15.11	22.04	6.93	0.693			0.693				
	O	10	10	14.12	21.02	6.90	0.690			0.690				
	P	10	10	13.99	22.45	8.46	0.846			0.846				
750	Q	10	8	14.72	20.82	6.10	0.763	0.825	7.3	0.610	77.5	0.638	6.2	29.1
	R	10	8	14.60	21.56	6.96	0.870			0.696				
	S	10	8	14.62	20.89	6.27	0.784			0.627				
	T	10	7	12.44	18.62	6.18	0.883			0.618				
900	U	10	3	14.67	17.19	2.52	0.840	0.900	7.1	0.252	40.0	0.364	27.4	59.6
	V	10	5	15.24	20.18	4.94	0.988			0.494				
	W	10	4	14.68	18.16	3.48	0.870			0.348				
	X	10	4	12.89	16.49	3.60	0.900			0.360				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.780	40.2	0.000	7.5	0.059	79.7	93.5
	Z	10	1	13.98	14.55	0.57	0.570			0.057				
	AA	10	1	12.57	13.20	0.63	0.630			0.063				
	BB	10	1	12.78	13.92	1.14	1.140			0.114				

Dunnett's MSD value: 0.1121 MSD = Minimum Significant Difference

PMSD: 12.5 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	7/12/2022	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	7/19/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	0.9000	1.0000	1.0000	1.0000
750	0.8000	0.8000	0.8000	0.7000
900	0.3000	0.5000	0.4000	0.4000
1050	0.0000	0.1000	0.1000	0.1000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.7750	0.7750	1.0782	0.9912	1.1071	5.379	4	10.00	10.00	9	40
*900	0.4000	0.4000	0.6836	0.5796	0.7854	12.289	4	10.00	10.00	24	40
*1050	0.0750	0.0750	0.2810	0.1588	0.3218	28.997	4	10.00	10.00	37	40

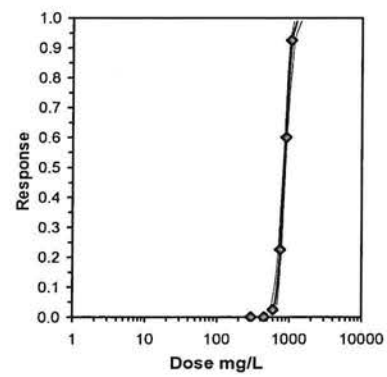
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.79483	0.896	-1.1515	1.61587
Equality of variance cannot be confirmed				

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

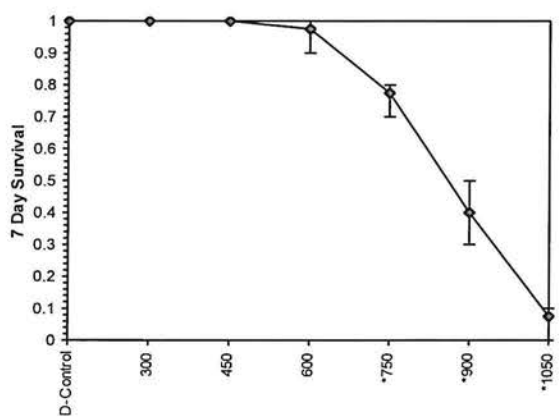
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.9761	1.84734	10.3553	17.5969	0	0.63606	9.48773	0.95897	2.92887	0.07155	3
Intercept	-35.934	5.41472	-46.547	-25.321							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	578.656	501.487	631.575
EC05	3.355	647.413	581.848	692.492
EC10	3.718	687.347	629.213	728.041
EC15	3.964	715.673	662.874	753.568
EC20	4.158	739.015	690.496	774.954
EC25	4.326	759.647	714.694	794.245
EC40	4.747	814.225	776.696	848.057
EC50	5.000	848.93	813.705	885.262
EC60	5.253	885.113	849.813	926.996
EC75	5.674	948.707	907.928	1006.82
EC80	5.842	975.192	930.671	1041.94
EC85	6.036	1007	957.21	1085.22
EC90	6.282	1048.5	990.889	1143.14
EC95	6.645	1113.17	1041.87	1236.06
EC99	7.326	1245.44	1142.48	1433.98



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Independent Review by Kelley E. Keenan:

ETS Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	7/12/2022	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	7/19/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	0.8860	0.8770	0.8440	0.9890
300	0.8230	0.7730	0.9330	0.8880
450	0.7820	0.9010	0.7830	0.7650
600	0.6790	0.6930	0.6900	0.8460
750	0.6100	0.6960	0.6270	0.6180
900	0.2520	0.4940	0.3480	0.3600
1050	0.0000	0.0570	0.0630	0.1140

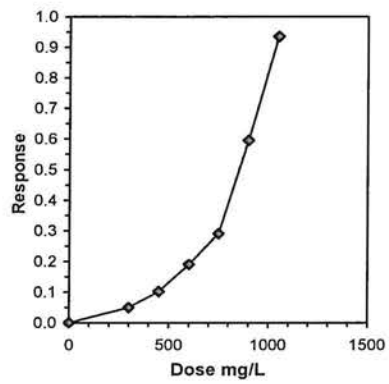
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.8990	1.0000	0.8990	0.8440	0.9890	6.970	4				0.8990	1.0000
300	0.8543	0.9502	0.8543	0.7730	0.9330	8.255	4	0.914	2.290	0.1121	0.8543	0.9502
450	0.8078	0.8985	0.8078	0.7650	0.9010	7.764	4	1.864	2.290	0.1121	0.8078	0.8985
*600	0.7270	0.8087	0.7270	0.6790	0.8460	10.944	4	3.514	2.290	0.1121	0.7270	0.8087
750	0.6378	0.7094	0.6378	0.6100	0.6960	6.186	4				0.6378	0.7094
900	0.3635	0.4043	0.3635	0.2520	0.4940	27.379	4				0.3635	0.4043
1050	0.0585	0.0651	0.0585	0.0000	0.1140	79.721	4				0.0585	0.0651

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.85182	0.844	0.86542	-0.6687
Bartlett's Test indicates equal variances (p = 0.98)	0.21125	11.3449		

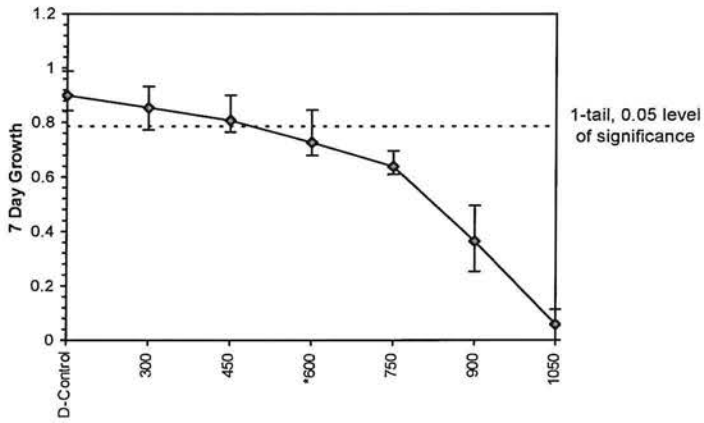
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	600	519.615		0.11208	0.12467	0.0216	0.00479	0.02444	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	300.65	105.03	0.00	556.96	-0.0326
IC10	445.65	89.72	90.99	671.24	-0.4411
IC15	530.99	67.19	326.39	714.72	-0.3414
IC20	613.11	52.82	450.69	745.60	-0.0314
IC25	688.66	48.62	516.02	793.73	-0.4629
IC40	803.79	15.02	762.04	861.90	0.9345
IC50	852.96	19.20	802.10	930.71	0.8506



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Independent Review by Kelley E. Keenan:

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **91**

Daily Chemistry:

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

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	K	K	K	K	K	K
Concentration	Parameter			7.39			
CONTROL, MHSW	pH (S.U.)	7.39	7.50	7.39	7.52	7.49	7.41
	Dissolved oxygen (mg/L)	7.6	7.0	7.9	7.9	7.6	6.0
	Conductivity (µmhos/cm)	293		302		313	
	Alkalinity (mg $CaCO_3$/L)	59				59	
	Hardness (mg $CaCO_3$/L)	86				86	
	Temperature (°C)	24.9	25.0	24.8	24.8	24.8	24.7
300 mg KCl/L	pH (S.U.)	7.69	7.57	7.71	7.59	7.74	7.43
	Dissolved oxygen (mg/L)	7.7	7.7	8.0	7.9	8.0	6.9
	Conductivity (µmhos/cm)	798		830		836	
	Temperature (°C)	24.8	24.9	24.7	25.0	24.9	24.7
450 mg KCl/L	pH (S.U.)	7.69	7.52	7.73	7.61	7.75	7.43
	Dissolved oxygen (mg/L)	7.7	7.7	8.0	7.8	8.0	6.9
	Conductivity (µmhos/cm)	1040		1060		1080	
	Temperature (°C)	24.8	24.9	24.8	24.7	24.9	24.9
600 mg KCl/L	pH (S.U.)	7.70	7.55	7.74	7.66	7.76	7.44
	Dissolved oxygen (mg/L)	7.7	7.8	8.0	7.8	8.1	6.8
	Conductivity (µmhos/cm)	1280		1320		1320	
	Temperature (°C)	24.8	25.1	24.8	24.7	25.0	25.0
750 mg KCl/L	pH (S.U.)	7.71	7.55	7.72	7.66	7.79	7.46
	Dissolved oxygen (mg/L)	7.7	7.8	8.0	7.8	8.0	6.8
	Conductivity (µmhos/cm)	1540		1540		1570	
	Temperature (°C)	24.9	24.8	24.7	24.9	25.0	25.0
900 mg KCl/L	pH (S.U.)	7.74	7.56	7.75	7.67	7.80	7.48
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.9	8.0	6.8
	Conductivity (µmhos/cm)	1780		1820		1840	
	Temperature (°C)	24.9	24.8	24.7	24.9	24.9	24.8
1050 mg KCl/L	pH (S.U.)	7.76	7.61	7.77	7.72	7.82	7.52
	Dissolved oxygen (mg/L)	7.9	7.9	8.0	7.8	8.0	6.6
	Conductivity (µmhos/cm)	2020		2080		2100	
	Temperature (°C)	25.0	24.8	24.7	24.8	24.9	25.0
		Initial	Final	Initial	Final	Initial	Final

Independent Review by Kelley E. Keenan: K

Environmental Testing Solutions, Inc.

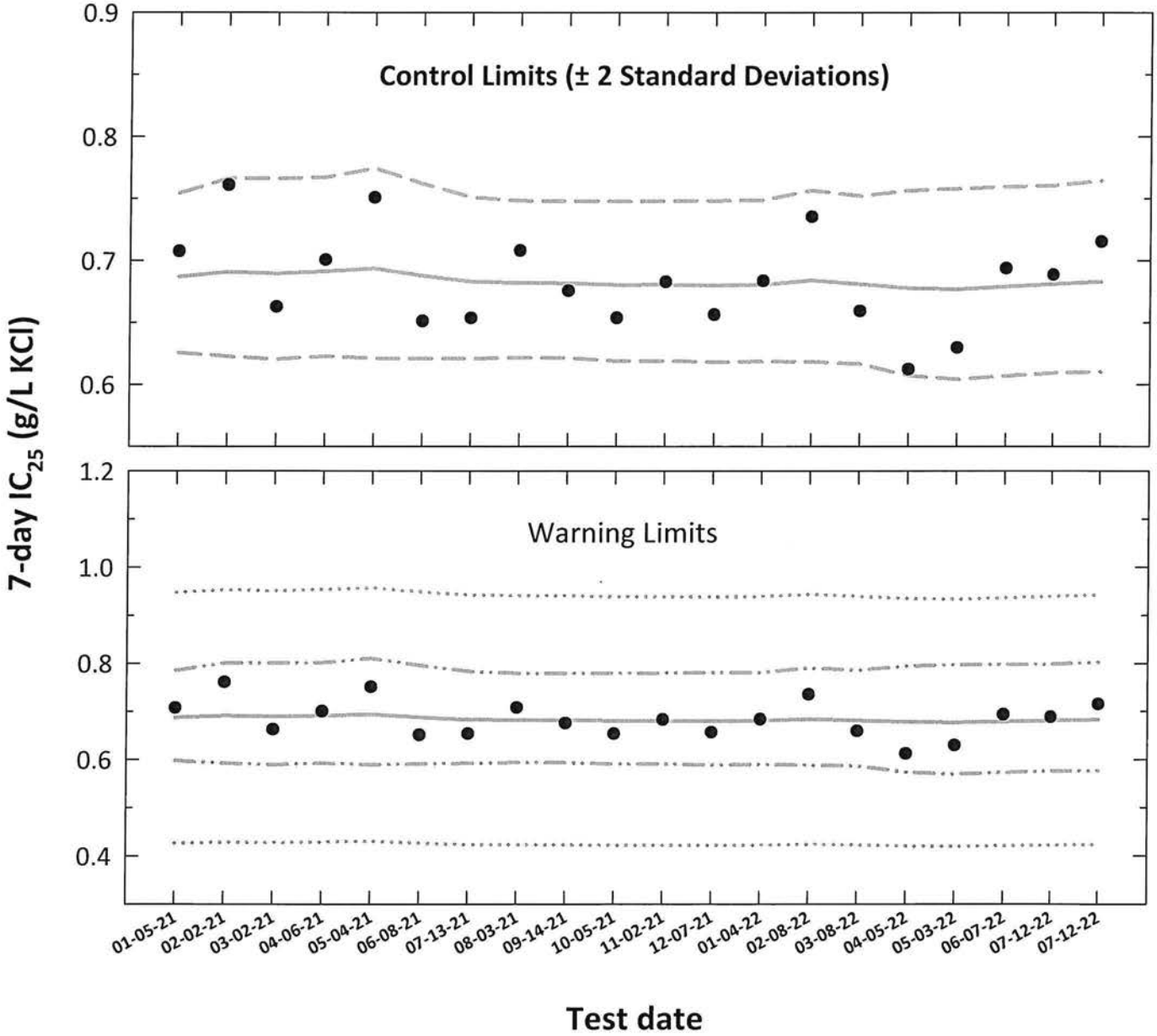
Species: ***Pimephales promelas*** PpKClCR Test Number: **91**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	KL	BSL	BSL	BSL	BSL	TG	TG	KL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.36	7.38	7.31	7.40	7.30	7.43	7.60 ~~TG 7.19~~	7.41
	Dissolved oxygen (mg/L)	7.6	7.5	7.7	7.3	7.7	7.5	7.7 ~~7.5~~	7.0
	Conductivity (µmhos/cm)	290		294		303		310	
	Alkalinity (mg $CaCO_3$/L)			59					
	Hardness (mg $CaCO_3$/L)			86					
	Temperature (°C)	24.8	24.8	24.8	24.7	24.8	24.8	24.8	24.8
300 mg KCl/L	pH (S.U.)	7.75	7.49	* ~~7.9~~ 7.78	7.51	7.74	~~7.51~~	~~7.52~~	7.45
	Dissolved oxygen (mg/L)	7.7	7.4	7.9	7.3	7.7	7.80 7.6	7.51 7.6	6.9
	Conductivity (µmhos/cm)	800		780		841		860	
	Temperature (°C)	24.8	24.9	24.8	24.9	24.7	24.6	24.8	24.5
450 mg KCl/L	pH (S.U.)	7.75	7.54	* ~~7.9~~ 7.83	7.56	7.74	7.63	7.59	7.51
	Dissolved oxygen (mg/L)	7.7	7.3	7.9	7.3	7.8	7.5	7.7	7.0
	Conductivity (µmhos/cm)	1020		1020		1060		1070	
	Temperature (°C)	24.9	24.7	24.8	24.6	24.8	24.6	24.8	24.7
600 mg KCl/L	pH (S.U.)	7.76	7.55	* ~~7.9~~ 7.86	7.54	7.78	7.66	7.64	7.55
	Dissolved oxygen (mg/L)	7.8	7.4	7.9	6.8	7.8	7.5	7.7	7.1
	Conductivity (µmhos/cm)	1270		1280		1330		1340	
	Temperature (°C)	24.9	24.7	24.9	24.6	24.7	24.6	24.8	24.7
750 mg KCl/L	pH (S.U.)	7.77	7.55	* ~~7.9~~ 7.89	7.56	7.79	7.67	7.71	7.57
	Dissolved oxygen (mg/L)	7.8	7.3	7.9	6.9	7.8	7.6	7.9	7.0
	Conductivity (µmhos/cm)	1510		1540		1570		1580	
	Temperature (°C)	24.8	24.9	24.9	24.6	24.7	24.6	24.8	24.7
900 mg KCl/L	pH (S.U.)	7.78	7.58	* ~~7.9~~ 7.90	7.57	7.82	7.76	7.73	7.58
	Dissolved oxygen (mg/L)	7.9	7.3	7.9	7.1	7.8	7.6	7.6	7.0
	Conductivity (µmhos/cm)	1770		1750		1820		1790	
	Temperature (°C)	24.9	24.9	24.9	24.8	24.7	24.6	24.9	24.6
1050 mg KCl/L	pH (S.U.)	7.81	7.59	* ~~8.0~~ 7.90	7.63	7.82	7.79	7.71	7.64
	Dissolved oxygen (mg/L)	7.8	7.3	8.0	7.4	7.8	7.6	7.7	6.9
	Conductivity (µmhos/cm)	2020		2000		2080		2070	
	Temperature (°C)	24.9	25.0	24.9	24.7	24.9	24.8	24.9	24.6
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Independent Review by Kelley E. Keenan: K

* wrote DO results in pH row - BSL 07.16.22

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	01-05-21	0.7076	-0.1502	-0.1630	0.0203	0.6870	0.6257	0.7544	0.5977	0.7850	0.4259	0.9481
2	02-02-21	0.7611	-0.1186	-0.1606	0.0226	0.6909	0.6228	0.7666	0.5923	0.8004	0.4284	0.9535
3	03-02-21	0.6628	-0.1786	-0.1615	0.0229	0.6895	0.6205	0.7662	0.5894	0.8008	0.4275	0.9515
4	04-06-21	0.7006	-0.1545	-0.1603	0.0226	0.6914	0.6230	0.7672	0.5925	0.8011	0.4287	0.9541
5	05-04-21	0.7509	-0.1244	-0.1589	0.0240	0.6937	0.6212	0.7746	0.5892	0.8103	0.4301	0.9573
6	06-08-21	0.6512	-0.1863	-0.1625	0.0222	0.6879	0.6210	0.7620	0.5907	0.7956	0.4265	0.9493
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

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

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

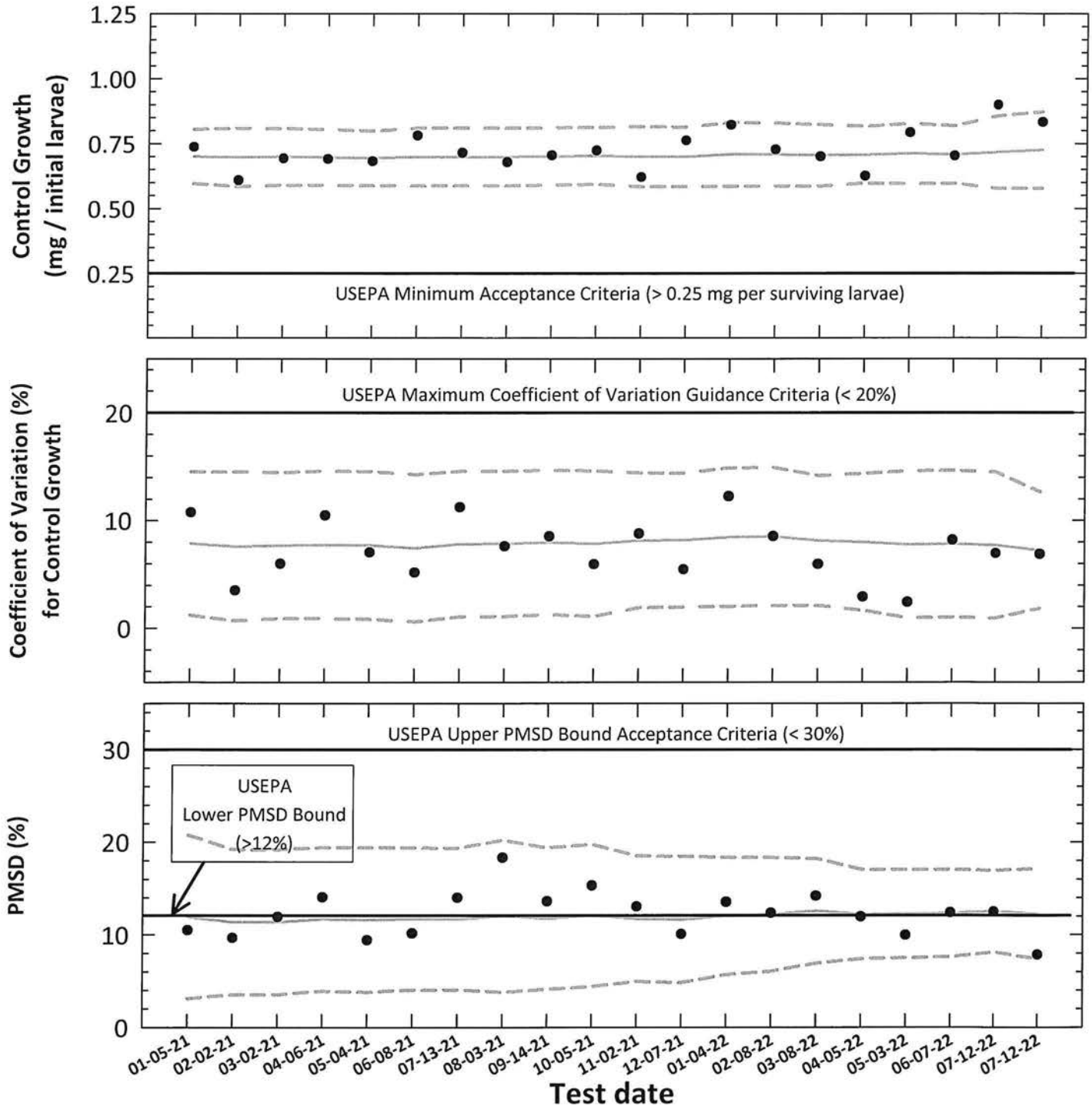
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

Central Tendency (mean Control Growth, CV or PMSD)

95% Confidence Interval (mean Control Growth, CV or PMSD ± 2 Standard Deviations)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival (%)	Control Growth Mean (mg/initial larvae)	Control Growth CV (%)	Test MSD	Test 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
1	01-05-21	100	0.737	10.8	0.0774	10.5	0.700	0.596	0.805	7.9	1.2	14.5	11.9	3.1	20.8
2	02-02-21	100	0.609	3.5	0.0588	9.7	0.697	0.585	0.809	7.6	0.7	14.5	11.4	3.5	19.2
3	03-02-21	100	0.693	6.0	0.0827	11.9	0.699	0.590	0.809	7.7	0.9	14.5	11.4	3.5	19.2
4	04-06-21	100	0.691	10.5	0.0970	14.0	0.697	0.589	0.805	7.7	0.9	14.6	11.7	3.9	19.4
5	05-04-21	100	0.682	7.1	0.0643	9.4	0.694	0.589	0.799	7.7	0.8	14.6	11.6	3.8	19.4
6	06-08-21	100	0.781	5.2	0.0794	10.2	0.699	0.587	0.811	7.4	0.6	14.3	11.7	4.0	19.4
7	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	0.810	7.8	1.1	14.6	11.7	4.0	19.3
8	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	1.1	14.6	12.0	3.8	20.2
9	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	1.3	14.7	11.7	4.1	19.4
10	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	14.6	12.1	4.4	19.7
11	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	14.4	11.7	4.9	18.5
12	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5
13	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3
14	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3
15	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2
16	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0
17	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	14.6	12.3	7.5	17.0
18	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	14.7	12.3	7.6	17.0
19	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	14.5	12.5	8.1	16.9
20	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.7	12.2	7.4	17.1

Note:

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

Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.

CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 12%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: **92 - New Artemia**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2101				
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:	BLUE
Age:	< 24-hours old	Incubator number and shelf location:	7C
Spawn date:	07-05-22	*Artemia* CHM number:	
Hatch dates and times:	07-11-22 1511 TO 07-12-22 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.04 S.U. Temperature = 24.5 °C	Date / Time in oven:	07-19-22 0646
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	07-20-22 0650
		*Final oven temperature:	60 °C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	07-12-22	0505	JL	1110	JL	0715	JL	07-07-22 A
1	07-13-22	0500	JL	1100	JL	0708	JL	↓
2	07-14-22	0500	JL	1100	JL	0700	JL	07-07-22 C
3	07-15-22	0500	JL	1100	JL	0700	JL	↓
4	07-16-22	0600	JL	1200	JL	0800	JL	07-12-22 A
5	07-17-22	0500	JL	1100	JL	0700	JL	↓
6	07-18-22	0500	JL	1100	JL	0700	JL	
7	07-19-22					0615	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	13066468S

Control information:		Acceptance criteria
% Mortality:	0%	≤ 20%
Average weight per initial larvae:	0.833	
Average weight per surviving larvae:	0.833	≥ 0.25 mg/larvae

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	874.9
NOEC (mg/L KCl)	600
LOEC (mg/L KCl)	750
ChV (mg/L KCl)	670.8
IC_{25} (mg/L KCl)	715.3

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **92 - New Artemia**

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: Grey Analyst: JR Date: 06-28-22	14.55	15.37	14.54	13.37	13.10	12.20	14.36	13.64	15.12	14.02	15.83	14.71
*B = Pan + Larvae weight (mg) Analyst: JR Date: 07-23-22	22.08	24.17	23.22	21.67	21.85	20.85	23.03	22.37	23.85	21.72	23.77	23.29
C = Larvae weight (mg) = B – A Analyst: [illegible]	7.53	8.80	8.68	8.30	8.75	8.65	8.67	8.73	8.73	7.70	7.94	8.58
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: [illegible]	0.753	0.880	0.868	0.830	0.875	0.865	0.867	0.873	0.873	0.770	0.794	0.858
Average weight per initial number of larvae (mg)	0.833				0.870				0.824			
Percent reduction from control (%)					-4.5%				1.1%			

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **92 - New Artemia**

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	9 1d	10	7 3d	7 3d	7 3d	7 3d
2	10	10	10	10	9	9	9	9 1d	7	7	6 1d	7
3	10	10	10	10	9	9	9	9	7	7	6	7
4	10	10	10	10	9	9	9	8 1d	6 1d	6 1d	6	6 1d
5	10	10	10	10	8 1d	8 1d	9	8	4 2d	5 1d	5 1d	5 1d
6	10	10	10	10	8	8	9	8	4 1d	5	4 1d	5
7	10	10	10	10	8	8	9	8	4	5	4	4 1d
*A = Pan weight (mg) Tray color code: Grey Analyst: JR Date: 06-28-22	14.88	13.48	14.57	14.53	13.77	12.88	15.61	14.14	13.23	13.31	14.53	12.60
*B = Pan + Larvae weight (mg) Analyst: JB Date: 07-23-22	22.80	21.35	21.90	22.11	20.46	18.30	21.77	19.86	17.01	17.47	17.61	16.26
C = Larvae weight (mg) = B – A Analyst: JA	7.92	7.87	7.33	7.58	6.69	5.42	6.16	5.72	3.78	4.16	3.08	3.66
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JA	0.792	0.787	0.733	0.758	0.669	0.542	0.616	0.572	0.378	0.416	0.308	0.366
Average weight per initial number of larvae (mg)	0.768				0.600				0.367			
Percent reduction from control (%)		7.8%				28.0%				55.9%		

*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:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **92 - New Artemia**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	4 cod	3 7d	3 7d	3 7d
2	3 1d	3	3	2 1d
3	2 1d	3	3	2
4	2	2 1d	2 1d	2
5	2	2	2	2
6	2	1 1d	1 1d	2
7	2	1	1	1 1d
*A = Pan weight (mg) Tray color code: Grey Analyst: JR Date: 06-28-22	13.27	14.09	15.32	13.35
*B = Pan + Larvae weight (mg) Analyst: JR Date: 07-23-22	14.69	14.87	16.01	14.38
C = Larvae weight (mg) = B – A Analyst: JR	1.42	0.78	0.69	1.03
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JR	0.142	0.078	0.069	0.103
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.098		88.27	

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

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

Comments:

Environmental Testing Solutions, Inc.

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: 92 - New Artemia

Test dates: July 12-19, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.55	22.08	7.53	0.753	0.833	6.9	0.753	100.0	0.833	6.9	Not applicable
	B	10	10	15.37	24.17	8.80	0.880			0.880				
	C	10	10	14.54	23.22	8.68	0.868			0.868				
	D	10	10	13.37	21.67	8.30	0.830			0.830				
300	E	10	10	13.10	21.85	8.75	0.875	0.870	0.5	0.875	100.0	0.870	0.5	-4.5
	F	10	10	12.20	20.85	8.65	0.865			0.865				
	G	10	10	14.36	23.03	8.67	0.867			0.867				
	H	10	10	13.64	22.37	8.73	0.873			0.873				
450	I	10	10	15.12	23.85	8.73	0.873	0.824	6.0	0.873	100.0	0.824	6.0	1.1
	J	10	10	14.02	21.72	7.70	0.770			0.770				
	K	10	10	15.83	23.77	7.94	0.794			0.794				
	L	10	10	14.71	23.29	8.58	0.858			0.858				
600	M	10	10	14.88	22.80	7.92	0.792	0.768	3.6	0.792	100.0	0.768	3.6	7.8
	N	10	10	13.48	21.35	7.87	0.787			0.787				
	O	10	10	14.57	21.90	7.33	0.733			0.733				
	P	10	10	14.53	22.11	7.58	0.758			0.758				
750	Q	10	8	13.77	20.46	6.69	0.836	0.728	10.1	0.669	82.5	0.600	9.2	28.0
	R	10	8	12.88	18.30	5.42	0.678			0.542				
	S	10	9	15.61	21.77	6.16	0.684			0.616				
	T	10	8	14.14	19.86	5.72	0.715			0.572				
900	U	10	4	13.23	17.01	3.78	0.945	0.866	9.2	0.378	42.5	0.367	12.2	55.9
	V	10	5	13.31	17.47	4.16	0.832			0.416				
	W	10	4	14.53	17.61	3.08	0.770			0.308				
	X	10	4	12.60	16.26	3.66	0.915			0.366				
1050	Y	10	2	13.27	14.69	1.42	0.710	0.803	19.5	0.142	12.5	0.098	33.3	88.2
	Z	10	1	14.09	14.87	0.78	0.780			0.078				
	AA	10	1	15.32	16.01	0.69	0.690			0.069				
	BB	10	1	13.35	14.38	1.03	1.030			0.103				

Dunnett's MSD value: 0.0653 MSD = Minimum Significant Difference

PMSD: 7.8 PMSD = Percent Minimum Significant Difference

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

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

Reviewed and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	7/12/2022	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	7/19/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:	New Artemia				

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	1.0000
750	0.8000	0.8000	0.9000	0.8000
900	0.4000	0.5000	0.4000	0.4000
1050	0.2000	0.1000	0.1000	0.1000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*750	0.8250	0.8250	1.1426	1.1071	1.2490	6.209	4	10.00	10.00	7	40
*900	0.4250	0.4250	0.7099	0.6847	0.7854	7.091	4	10.00	10.00	23	40
*1050	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40

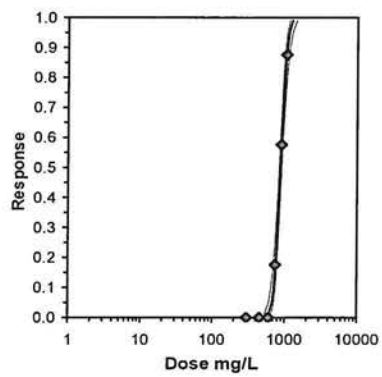
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.68081	0.896	1.92265	3.70996
Equality of variance cannot be confirmed				

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

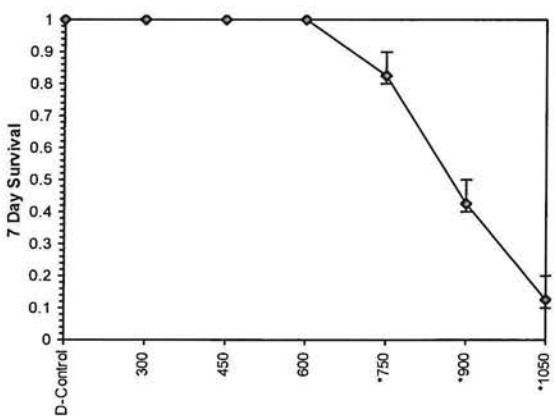
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.0038	2.06208	10.9621	19.0455	0	0.39483	9.48773	0.9829	2.94196	0.06665	3
Intercept	-39.141	6.06673	-51.031	-27.25							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	612.222	532.908	665.265
EC05	3.355	679.721	613.19	724.441
EC10	3.718	718.695	660.182	758.842
EC15	3.964	746.246	693.428	783.51
EC20	4.158	768.893	720.609	804.168
EC25	4.326	788.87	744.336	822.809
EC40	4.747	841.539	804.696	874.917
EC50	5.000	874.903	840.359	911.06
EC60	5.253	909.59	874.889	951.639
EC75	5.674	970.319	930.056	1029.07
EC80	5.842	995.529	951.552	1063.03
EC85	6.036	1025.74	976.585	1104.76
EC90	6.282	1065.06	1008.28	1160.45
EC95	6.645	1126.13	1056.11	1249.44
EC99	7.326	1250.29	1150	1437.73



Dose-Response Plot



Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	7/12/2022	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	7/19/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:	New Artemia				

Conc-mg/L	1	2	3	4
D-Control	0.7530	0.8800	0.8680	0.8300
300	0.8750	0.8650	0.8670	0.8730
450	0.8730	0.7700	0.7940	0.8580
600	0.7920	0.7870	0.7330	0.7580
750	0.6690	0.5420	0.6160	0.5720
900	0.3780	0.4160	0.3080	0.3660
1050	0.1420	0.0780	0.0690	0.1030

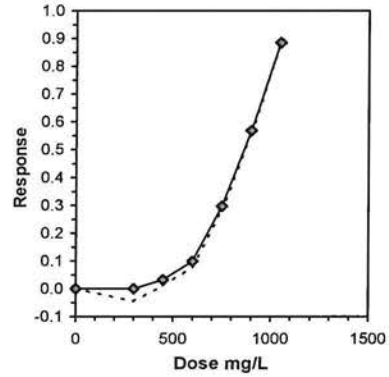
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.8328	1.0000	0.8328	0.7530	0.8800	6.878	4				0.8514	1.0000
300	0.8700	1.0447	0.8700	0.8650	0.8750	0.547	4	-1.305	2.290	0.0653	0.8514	1.0000
450	0.8238	0.9892	0.8238	0.7700	0.8730	6.018	4	0.315	2.290	0.0653	0.8238	0.9676
600	0.7675	0.9216	0.7675	0.7330	0.7920	3.577	4	2.287	2.290	0.0653	0.7675	0.9015
750	0.5998	0.7202	0.5998	0.5420	0.6690	9.216	4				0.5998	0.7044
900	0.3670	0.4407	0.3670	0.3080	0.4160	12.190	4				0.3670	0.4311
1050	0.0980	0.1177	0.0980	0.0690	0.1420	33.337	4				0.0980	0.1151

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95213	0.844	-0.6475	0.12341
Bartlett's Test indicates equal variances (p = 0.02)	10.3614	11.3449		

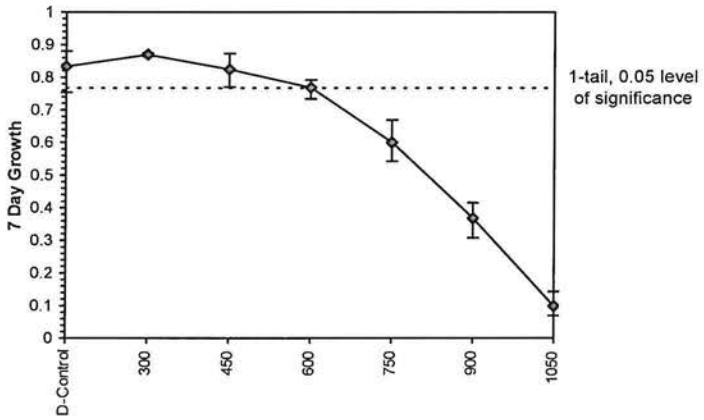
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.06535	0.07847	0.00718	0.00163	0.02614	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	489.85	45.33	331.26	613.42	-0.4272
IC10	601.13	24.23	491.81	640.00	-1.4689
IC15	639.19	13.28	599.90	685.68	-0.0870
IC20	677.26	15.31	638.03	734.50	0.4817
IC25	715.32	18.01	668.33	777.89	0.3005
IC40	807.31	11.54	768.26	837.73	-0.4003
IC50	862.18	10.39	834.85	897.45	0.2803



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **92 - New Artemia**

Daily Chemistry:

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

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	K	K	K	K	K	K
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.39	7.46	7.39	7.52	7.49	7.25
	Dissolved oxygen (mg/L)	7.6	7.6	7.9	7.9	7.6	6.4
	Conductivity (µmhos/cm)	293		302		313	
	Alkalinity (mg $CaCO_3$/L)	59				59	
	Hardness (mg $CaCO_3$/L)	86				86	
	Temperature (°C)	24.8	24.9	24.8	25.1	24.8	24.8
300 mg KCl/L	pH (S.U.)	7.69	7.50	7.71	7.58	7.74	7.41
	Dissolved oxygen (mg/L)	7.7	7.6	8.0	7.8	8.0	6.5
	Conductivity (µmhos/cm)	798		830		836	
	Temperature (°C)	24.9	25.1	24.7	24.8	24.7	25.1
450 mg KCl/L	pH (S.U.)	7.69	7.53	7.73	7.64	7.75	7.44
	Dissolved oxygen (mg/L)	7.7	7.7	8.0	7.7	8.0	6.7
	Conductivity (µmhos/cm)	1040		1060		1080	
	Temperature (°C)	24.9	24.8	24.7	24.7	24.9	24.8
600 mg KCl/L	pH (S.U.)	7.70	7.54	7.74	7.66	7.76	7.49
	Dissolved oxygen (mg/L)	7.7	7.7	8.0	7.7	8.1	6.8
	Conductivity (µmhos/cm)	1280		1320		1320	
	Temperature (°C)	24.9	25.0	24.7	24.8	24.9	24.8
750 mg KCl/L	pH (S.U.)	7.71	7.56	7.78	7.66	7.79	7.52
	Dissolved oxygen (mg/L)	7.7	7.8	8.0	7.8	8.0	6.8
	Conductivity (µmhos/cm)	1540		1540		1570	
	Temperature (°C)	24.9	24.9	24.7	24.8	24.9	24.8
900 mg KCl/L	pH (S.U.)	7.74	7.56	7.75	7.67	7.80	7.53
	Dissolved oxygen (mg/L)	7.8	7.8	8.0	7.8	8.0	7.2
	Conductivity (µmhos/cm)	1780		1820		1840	
	Temperature (°C)	24.9	24.9	24.8	24.9	24.8	24.9
1050 mg KCl/L	pH (S.U.)	7.76	7.61	7.77	7.73	7.82	7.52
	Dissolved oxygen (mg/L)	7.9	7.8	8.0	7.9	8.0	7.1
	Conductivity (µmhos/cm)	2020		2080		2100	
	Temperature (°C)	24.9	24.8	24.8	25.0	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final

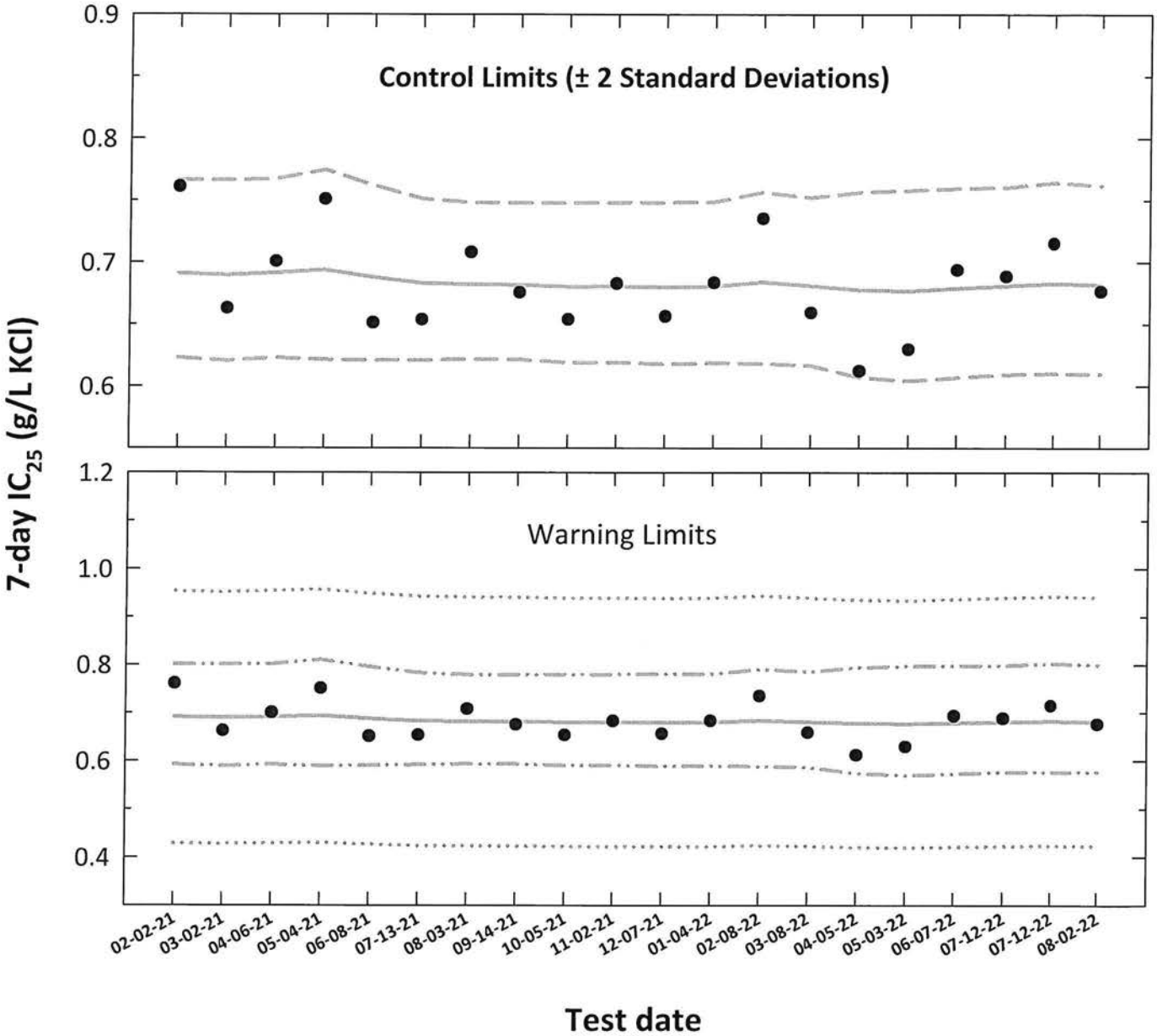
ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **92 - New Artemia**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	K	BSL	BSL	BSL	BSL	TG	TG	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.36	7.38	7.31	7.38	7.30	7.44	7.60	7.52
	Dissolved oxygen (mg/L)	7.6	7.5	7.7	7.3	7.7	7.4	~~7.4~~ 7.9	6.9
	Conductivity (µmhos/cm)	298		294		303		310	
	Alkalinity (mg $CaCO_3$/L)			59					
	Hardness (mg $CaCO_3$/L)			86					
	Temperature (°C)	24.8	24.9	24.8	24.8	24.8	24.8	24.8	24.6
300 mg KCl/L	pH (S.U.)	7.75	7.50	7.78	7.47	7.74	7.44	7.44	7.56
	Dissolved oxygen (mg/L)	7.7	7.4	7.9	7.3	7.8	7.5	7.5	7.0
	Conductivity (µmhos/cm)	806		780		841		830	
	Temperature (°C)	24.9	25.0	24.9	24.5	24.7	24.7	24.9	24.5
450 mg KCl/L	pH (S.U.)	7.75	~~7.58~~ 7.48	7.83	7.51	7.74	7.54	7.77	7.58
	Dissolved oxygen (mg/L)	7.7	7.3	7.9	* ~~7.5~~ 7.0	7.8	7.4	7.7	7.0
	Conductivity (µmhos/cm)	1020		1020		1060		1070	
	Temperature (°C)	24.8	24.7	24.9	24.7	24.7	24.7	24.9	24.8
600 mg KCl/L	pH (S.U.)	7.76	7.54	7.86	7.56	7.78	7.56	7.73	7.60
	Dissolved oxygen (mg/L)	7.8	7.3	7.9	7.1	7.8	7.6	7.7	7.0
	Conductivity (µmhos/cm)	1270		1280		1330		1290	
	Temperature (°C)	24.8	24.7	24.8	24.6	24.7	24.7	24.8	24.8
750 mg KCl/L	pH (S.U.)	7.77	7.63	7.89	7.58	7.79	7.58	7.81	7.61
	Dissolved oxygen (mg/L)	7.8	7.2	7.9	7.2	7.8	7.5	7.7	7.0
	Conductivity (µmhos/cm)	1510		1540		1570		1580	
	Temperature (°C)	24.8	24.9	24.8	24.6	24.7	24.7	24.8	24.6
900 mg KCl/L	pH (S.U.)	7.78	7.64	7.90	* ~~7.70~~ 7.59	7.82	7.62	7.78	7.63
	Dissolved oxygen (mg/L)	7.9	7.4	7.9	7.3	7.8	7.6	7.6	7.0
	Conductivity (µmhos/cm)	1770		1750		1820		1840	
	Temperature (°C)	24.9	24.9	24.8	24.6	24.7	24.6	24.8	24.6
1050 mg KCl/L	pH (S.U.)	7.81	7.69	7.90	* ~~7.72~~ 7.62	7.82	7.64	7.73	7.64
	Dissolved oxygen (mg/L)	7.8	7.3	8.0	7.1	7.8	7.5	7.7	6.8
	Conductivity (µmhos/cm)	2020		2000		2080		2090	
	Temperature (°C)	24.9	24.8	24.8	24.7	24.8	24.6	24.8	24.7
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

* BSL 7.17.22

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	02-02-21	0.7611	-0.1186	-0.1606	0.0226	0.6909	0.6228	0.7666	0.5923	0.8004	0.4284	0.9535
2	03-02-21	0.6628	-0.1786	-0.1615	0.0229	0.6895	0.6205	0.7662	0.5894	0.8008	0.4275	0.9515
3	04-06-21	0.7006	-0.1545	-0.1603	0.0226	0.6914	0.6230	0.7672	0.5925	0.8011	0.4287	0.9541
4	05-04-21	0.7509	-0.1244	-0.1589	0.0240	0.6937	0.6212	0.7746	0.5892	0.8103	0.4301	0.9573
5	06-08-21	0.6512	-0.1863	-0.1625	0.0222	0.6879	0.6210	0.7620	0.5907	0.7956	0.4265	0.9493
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

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

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

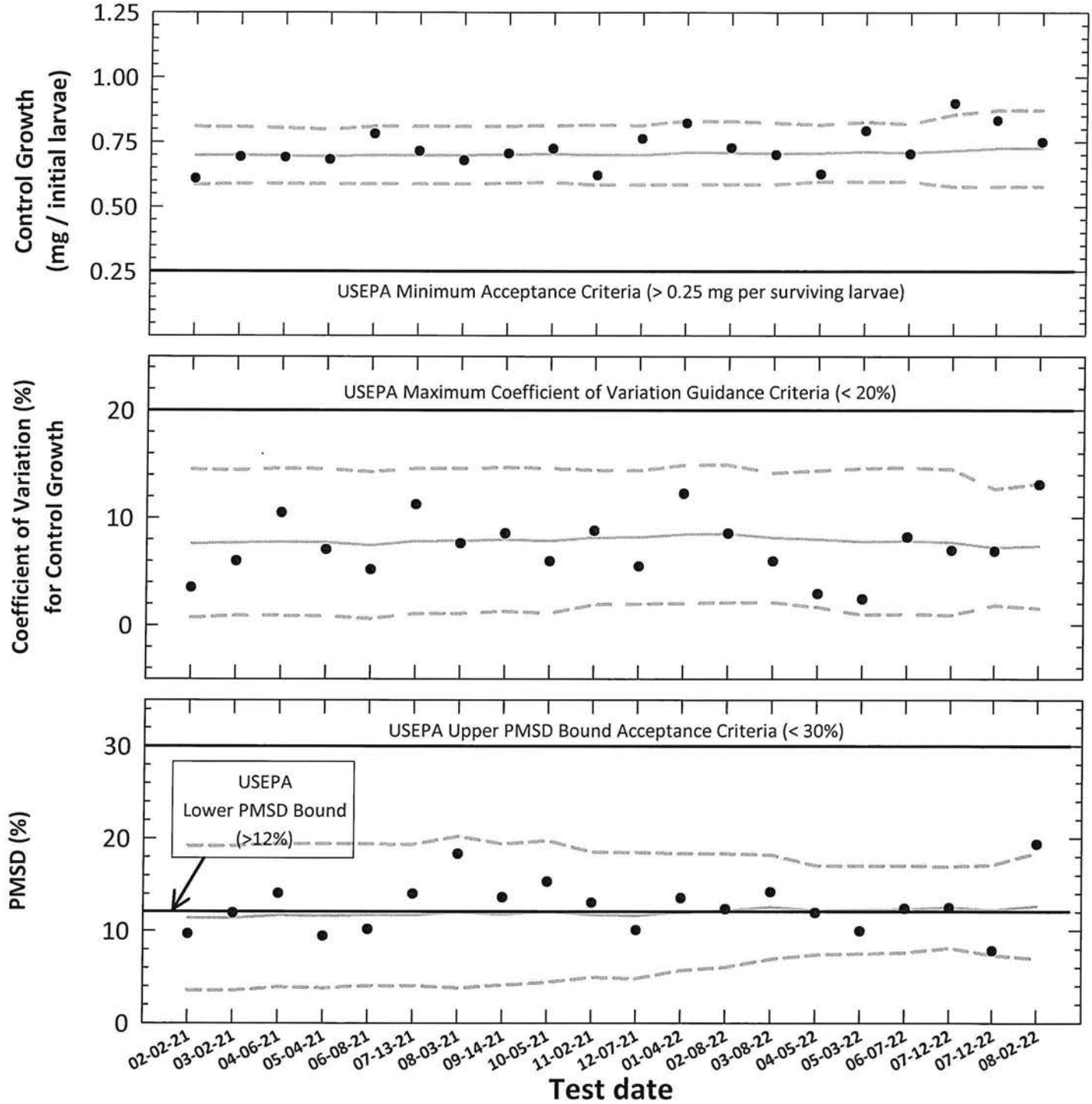
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

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

- - - - **95% Confidence Interval** (mean Control Growth, CV or PMSD ± 2 Standard Deviations)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival (%)	Control Growth Mean (mg/initial larvae)	Control Growth CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S
1	02-02-21	100	0.609	3.5	0.0588	9.7	0.697	0.585	0.809	7.6	0.7	14.5	11.4	3.5	19.2
2	03-02-21	100	0.693	6.0	0.0827	11.9	0.699	0.590	0.809	7.7	0.9	14.5	11.4	3.5	19.2
3	04-06-21	100	0.691	10.5	0.0970	14.0	0.697	0.589	0.805	7.7	0.9	14.6	11.7	3.9	19.4
4	05-04-21	100	0.682	7.1	0.0643	9.4	0.694	0.589	0.799	7.7	0.8	14.6	11.6	3.8	19.4
5	06-08-21	100	0.781	5.2	0.0794	10.2	0.699	0.587	0.811	7.4	0.6	14.3	11.7	4.0	19.4
6	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	0.810	7.8	1.1	14.6	11.7	4.0	19.3
7	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	1.1	14.6	12.0	3.8	20.2
8	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	1.3	14.7	11.7	4.1	19.4
9	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	14.6	12.1	4.4	19.7
10	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	14.4	11.7	4.9	18.5
11	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5
12	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3
13	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3
14	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2
15	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0
16	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	14.6	12.3	7.5	17.0
17	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	14.7	12.3	7.6	17.0
18	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	14.5	12.5	8.1	16.9
19	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.7	12.2	7.4	17.1
20	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	1.5	13.2	12.7	6.9	18.4

Note:

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

Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.

CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 12%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: 93

Dilution preparation information:							*Comments:*
KCl Stock INSS number:			INSS 2118				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		*Test information:*	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	07-26-22	*Artemia* CHM number:	CHM1149
Hatch dates and times:	08-01-22 1502 TO 08-02-22 0500	*Drying information for weight determination:*	
Transfer vessel information:	pH = 7.92 S.U. Temperature = 24.6 °C	Date / Time in oven:	08-09-22 0650
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	08-10-22 0650
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	08-02-22	0505	JK	1145	JK	0714	JK	07-29-22 B
1	08-03-22	0500	JK	1100	JK	0709	JK	↓
2	08-04-22	0500	JK	1100	JK	0710	JK	07-29-22 D
3	08-05-22	0500	JK	1100	JK	0700	JK	↓
4	08-06-22	0610	JK	1210	JK	0810	JK	07-29-22 E
5	08-07-22	0600	JK	1200	JK	0800	JK	↓
6	08-08-22	0600	JK	1200	JK	0800	JK	↓
7	08-09-22					0615	JK	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2011	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O G-2016	YSI Model 52CE	18D104324
Conductivity	14.9 µmhos/cm	SM 2510 B-2011	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2011	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2011	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	*Summary of test endpoints:*	
% Mortality:	0 %	≤ 20%	**7-day LC_{50}** (mg/L KCl)	503.5
Average weight per initial larvae:	0.750		**NOEC** (mg/L KCl)	450
Average weight per surviving larvae:	0.750	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	600
			ChV (mg/L KCl)	519.6
			IC_{25} (mg/L KCl)	676.6

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **93**

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10 lg	10	10	10	10	10	10 lg	10 lg	10	10	10 lg
*A = Pan weight (mg) Tray color code: Forest green Analyst: JR Date: 07-26-22	14.62	14.10	14.91	15.82	12.59	15.20	14.58	14.54	14.27	14.96	14.60	14.31
*B = Pan + Larvae weight (mg) Analyst: EC Date: 08-11-22	22.37	22.87	21.53	22.66	19.62	21.90	21.54	22.78	22.64	21.17	22.23	23.02
C = Larvae weight (mg) = B – A Analyst: JI	7.75	8.77	6.62	6.84	7.03	6.70	6.96	8.24	8.37	6.21	7.63	8.71
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JI	0.775	0.877	0.662	0.684	0.703	0.670	0.696	0.824	0.837	0.621	0.763	0.871
Average weight per initial number of larvae (mg)	0.750				0.723				0.773			
Percent reduction from control (%)					3.5%				-3.1%			

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **93**

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	8 2d	8 2d	8 2d	4 6d	5 5d	4 6d	5 5d
2	10	10	10	10	10	8	8	8	4	5	3 1d	5
3	10	10	10	10	9 1d	8	8	8	4	5	3	5
4	10	10	10	10	9	8	8	8	4	5	3	4 1d
5	10	10	9 1d	9 1d	8 1d	8	8	7 1d	3 1d	5	3	3 1d
6	10	10	9	9	8	8	7 1d	7	3	5	3	3
7	9 1d	9 1d	9 1d	9	7 1d	7 1d	7	6 1d	3 1d	4 1d	3 1d	3
*A = Pan weight (mg) Tray color code: Forest green Analyst: JR Date: 07-26-22	15.76	14.94	13.48	15.17	12.81	13.37	14.53	14.98	13.12	15.15	13.52	14.74
*B = Pan + Larvae weight (mg) Analyst: EC Date: 08-11-22	22.30	20.63	20.85	21.26	17.36	18.91	20.05	18.78	15.82	18.02	16.10	17.13
C = Larvae weight (mg) = B – A Analyst: JL	6.54	5.69	7.37	6.09	4.55	5.54	5.52	3.80	2.70	2.87	2.58	2.39
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.654	0.569	0.737	0.609	0.455	0.554	0.552	0.380	0.270	0.287	0.258	0.239
Average weight per initial number of larvae (mg)	0.642				0.485				0.264			
Percent reduction from control (%)		14.3%				35.3%				64.8%		

*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:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **93**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 7d	2 8d	2 8d	3 7d
2	3	2	2	2 1d
3	3	2	2	2
4	2 1d	1 1d	2	1 1d
5	1 1d	1	1 1d	1
6	1	1	1	0 1d
7	1	1	0 1d	0
*A = Pan weight (mg) Tray color code: forestgreen Analyst: JR Date: 07-26-22	13.38	14.24	~~15.55~~	~~13.68~~ 08-16-22 JR
*B = Pan + Larvae weight (mg) Analyst: ΣC Date: 08-11-22	14.06	14.99	~~15.55~~	~~13.67~~ 08-16-22 JR
C = Larvae weight (mg) = B – A Analyst: JR	0.68	0.75	—	— 08-16-22 JR
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JR	0.068	0.075	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.036		95.27	

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

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

Comments:

Environmental Testing Solutions, Inc.

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: 93

Test dates: August 02-09, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	14.62	22.37	7.75	0.775	0.750	13.1	0.775	100.0	0.750	13.1	Not applicable
	B	10	10	14.10	22.87	8.77	0.877			0.877				
	C	10	10	14.91	21.53	6.62	0.662			0.662				
	D	10	10	15.82	22.66	6.84	0.684			0.684				
300	E	10	10	12.59	19.62	7.03	0.703	0.723	9.5	0.703	100.0	0.723	9.5	3.5
	F	10	10	15.20	21.90	6.70	0.670			0.670				
	G	10	10	14.58	21.54	6.96	0.696			0.696				
	H	10	10	14.54	22.78	8.24	0.824			0.824				
450	I	10	10	14.27	22.64	8.37	0.837	0.773	14.3	0.837	100.0	0.773	14.3	-3.1
	J	10	10	14.96	21.17	6.21	0.621			0.621				
	K	10	10	14.60	22.23	7.63	0.763			0.763				
	L	10	10	14.31	23.02	8.71	0.871			0.871				
600	M	10	9	15.76	22.30	6.54	0.727	0.714	11.2	0.654	90.0	0.642	11.2	14.3
	N	10	9	14.94	20.63	5.69	0.632			0.569				
	O	10	9	13.48	20.85	7.37	0.819			0.737				
	P	10	9	15.17	21.26	6.09	0.677			0.609				
750	Q	10	7	12.81	17.36	4.55	0.650	0.716	12.0	0.455	67.5	0.485	17.3	35.3
	R	10	7	13.37	18.91	5.54	0.791			0.554				
	S	10	7	14.53	20.05	5.52	0.789			0.552				
	T	10	6	14.98	18.78	3.80	0.633			0.380				
900	U	10	3	13.12	15.82	2.70	0.900	0.819	9.7	0.270	32.5	0.264	7.7	64.8
	V	10	4	15.15	18.02	2.87	0.718			0.287				
	W	10	3	13.52	16.10	2.58	0.860			0.258				
	X	10	3	14.74	17.13	2.39	0.797			0.239				
1050	Y	10	1	13.38	14.06	0.68	0.680	0.715	6.9	0.068	5.0	0.036	115.7	95.2
	Z	10	1	14.24	14.99	0.75	0.750			0.075				
	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: 0.1452 MSD = Minimum Significant Difference

PMSD: 19.4 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by JH Summer

Environmental Testing Solutions, Inc.

Statistical Analyses

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	8/2/2022	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	8/9/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	0.9000	0.9000	0.9000	0.9000
750	0.7000	0.7000	0.7000	0.6000
900	0.3000	0.4000	0.3000	0.3000
1050	0.1000	0.1000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*600	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	4	10.00	10.00	4	40
*750	0.6750	0.6750	0.9649	0.8861	0.9912	5.445	4	10.00	10.00	13	40
*900	0.3250	0.3250	0.6059	0.5796	0.6847	8.671	4	10.00	10.00	27	40
*1050	0.0500	0.0500	0.2403	0.1588	0.3218	39.161	4	10.00	10.00	38	40

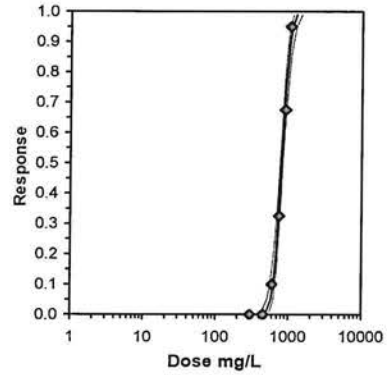
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.82248	0.896	1.8E-15	1.28546
Equality of variance cannot be confirmed				

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

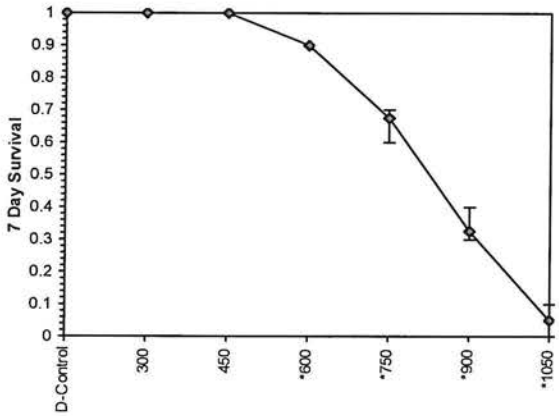
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.7055	1.46703	8.83008	14.5808	0	1.97084	9.48773	0.74112	2.90501	0.08543	3
Intercept	-29.004	4.26931	-37.372	-20.637							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	508.473	432.929	562.439
EC05	3.355	581.418	515.492	628.331
EC10	3.718	624.49	565.168	667.257
EC15	3.964	655.339	600.919	695.385
EC20	4.158	680.94	630.536	719.042
EC25	4.326	703.699	656.696	740.439
EC40	4.747	764.478	724.71	800.327
EC50	5.000	803.542	766.025	841.89
EC60	5.253	844.602	806.85	888.735
EC75	5.674	917.552	873.495	979.224
EC80	5.842	948.219	899.855	1019.44
EC85	6.036	985.262	930.798	1069.3
EC90	6.282	1033.93	970.34	1136.59
EC95	6.645	1110.53	1030.76	1245.75
EC99	7.326	1269.84	1151.83	1482.91



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	8/2/2022	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	8/9/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	0.7750	0.8770	0.6620	0.6840
300	0.7030	0.6700	0.6960	0.8240
450	0.8370	0.6210	0.7630	0.8710
600	0.6540	0.5690	0.7370	0.6090
750	0.4550	0.5540	0.5520	0.3800
900	0.2700	0.2870	0.2580	0.2390
1050	0.0680	0.0750	0.0000	0.0000

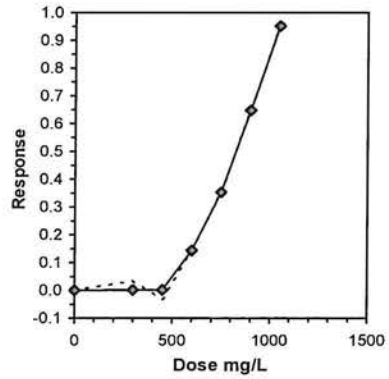
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7495	1.0000	0.7495	0.6620	0.8770	13.085	4				0.7495	1.0000
300	0.7233	0.9650	0.7233	0.6700	0.8240	9.492	4	0.394	2.180	0.1452	0.7481	0.9982
450	0.7730	1.0314	0.7730	0.6210	0.8710	14.348	4	-0.353	2.180	0.1452	0.7481	0.9982
600	0.6423	0.8569	0.6423	0.5690	0.7370	11.223	4				0.6423	0.8569
750	0.4853	0.6474	0.4853	0.3800	0.5540	17.313	4				0.4853	0.6474
900	0.2635	0.3516	0.2635	0.2390	0.2870	7.669	4				0.2635	0.3516
1050	0.0358	0.0477	0.0358	0.0000	0.0750	115.746	4				0.0358	0.0477

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96566	0.805	-0.0578	-0.7587
Bartlett's Test indicates equal variances (p = 0.74)	0.59281	9.21035		

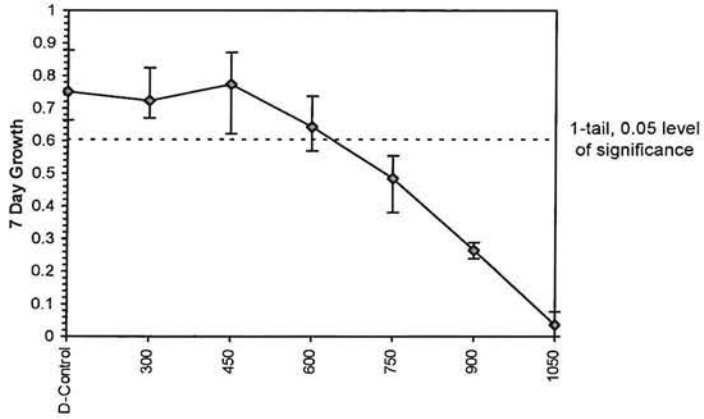
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	>450			0.14524	0.19378	0.00248	0.00888	0.76278	2, 9
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	501.15	133.22	0.00	642.77	-1.1667
IC10	554.24	79.30	90.10	668.00	-1.9312
IC15	604.94	45.90	411.53	679.54	-1.5733
IC20	640.75	35.76	501.75	706.22	-1.7190
IC25	676.55	31.38	559.53	760.30	-0.0831
IC40	774.05	23.93	687.87	820.37	-0.6367
IC50	824.75	18.17	751.15	855.27	-0.9410



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **93**

Daily Chemistry:

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

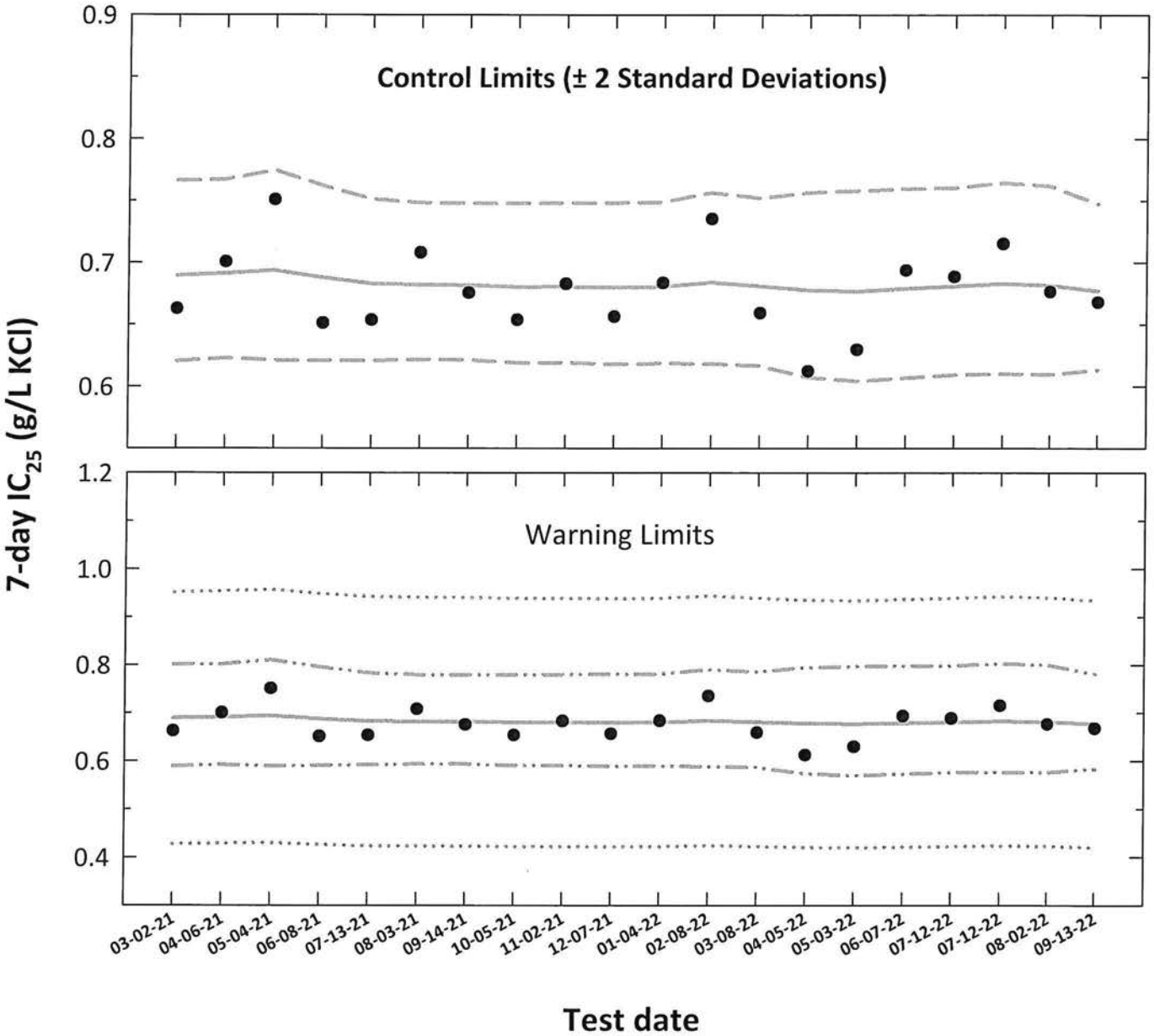
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	EC	EC M	EC M	EC	EC	M
Concentration	Parameter					080422 M	
CONTROL, MHSW	pH (S.U.)	7.06	7.17	7.13	7.45	~~7.77~~ 7.23	7.15
	Dissolved oxygen (mg/L)	7.6	7.7	7.5	7.6	7.7	6.7
	Conductivity (µmhos/cm)	300		314		306	
	Alkalinity (mg $CaCO_3$/L)	60				60	
	Hardness (mg $CaCO_3$/L)	86			08-01-22	90	
	Temperature (°C)	24.8	24.8	24.8	24.8	24.8	25.1
300 mg KCl/L	pH (S.U.)	7.61	~~8.43~~ 7.34	7.52	7.52	7.76	7.18
	Dissolved oxygen (mg/L)	7.6	7.7	7.6	7.7	7.7	6.8
	Conductivity (µmhos/cm)	847		843		850	
	Temperature (°C)	24.9	25.0	24.8	24.9	24.7	24.8
450 mg KCl/L	pH (S.U.)	7.63	7.37	7.56	7.51	7.76	7.19
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.6	7.8	6.7
	Conductivity (µmhos/cm)	1060		1090		1090	
	Temperature (°C)	24.9	24.8	24.7	24.7	24.7	24.8
600 mg KCl/L	pH (S.U.)	7.66	7.42	7.59	7.51	7.76	7.20
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.7	7.8	6.5
	Conductivity (µmhos/cm)	1350		1360		1360	
	Temperature (°C)	25.0	24.8	24.7	24.9	24.8	24.8
750 mg KCl/L	pH (S.U.)	7.67	7.45	7.62	7.55	7.76	7.23
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.7	7.9	7.0
	Conductivity (µmhos/cm)	1560		1560		1590	
	Temperature (°C)	25.0	24.9	24.7	24.9	24.8	24.7
900 mg KCl/L	pH (S.U.)	7.69	7.49	7.64	7.57	7.76	7.24
	Dissolved oxygen (mg/L)	7.8	7.7	7.8	7.6	7.9	7.0
	Conductivity (µmhos/cm)	1840		1840		1850	
	Temperature (°C)	25.0	24.7	24.7	24.9	24.8	24.9
1050 mg KCl/L	pH (S.U.)	7.71	7.53	7.65	7.59	7.76	7.27
	Dissolved oxygen (mg/L)	7.8	7.8	7.9	7.6	7.9	7.0
	Conductivity (µmhos/cm)	2070		2100		2120	
	Temperature (°C)	25.0	24.7	24.7	24.8	24.8	24.8
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **93**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	N	BS	BS	N	N	EC N	EC N	EC
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.63	7.03	7.09	7.35	7.70	7.14	7.50	7.21
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	6.9	7.6	7.4	7.7	7.5
	Conductivity (µmhos/cm)	297		293		305		300	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)		08.07.22	88				08.07.22	
	Temperature (°C)	24.8	24.9	24.8	24.9	24.8	24.9	24.8	24.8
300 mg KCl/L	pH (S.U.)	7.71	7.18	7.62	7.34	7.76	7.23	7.61	7.41
	Dissolved oxygen (mg/L)	7.7	7.4	8.0	6.6	7.6	7.4	7.9	7.4
	Conductivity (µmhos/cm)	822		809		832		829	
	Temperature (°C)	24.9	24.8	24.9	24.8	24.7	24.7	24.9	24.9
450 mg KCl/L	pH (S.U.)	7.69	7.32	7.65	7.35	7.76	7.28	7.63	7.45
	Dissolved oxygen (mg/L)	7.7	7.7	8.0	6.2	7.6	7.5	7.9	7.5
	Conductivity (µmhos/cm)	1060		1040		1090		1080	
	Temperature (°C)	24.9	25.0	24.9	24.8	24.7	24.7	24.8	24.7
600 mg KCl/L	pH (S.U.)	7.69	7.32	7.68	7.33	7.77	7.31	7.65	7.49
	Dissolved oxygen (mg/L)	7.8	7.6	8.0	6.2	7.7	7.6	7.9	7.6
	Conductivity (µmhos/cm)	1330		1270		1340		1320	
	Temperature (°C)	24.9	24.8	24.9	24.8	24.7	24.8	24.8	24.7
750 mg KCl/L	pH (S.U.)	7.69	7.34	7.68	7.34	7.77	7.33	7.67	7.52
	Dissolved oxygen (mg/L)	7.9	7.5	8.0	6.0	7.8	7.7	7.9	7.5
	Conductivity (µmhos/cm)	1560		1530		1580		1566	
	Temperature (°C)	24.8	24.8	24.9	24.8	24.8	25.0	24.8	24.9
900 mg KCl/L	pH (S.U.)	7.71	7.44	7.68	7.33	7.79	7.37	7.70	7.53
	Dissolved oxygen (mg/L)	8.0	7.7	8.0	6.0	7.9	7.7	8.0	7.5
	Conductivity (µmhos/cm)	1820		1780		1860		1830	
	Temperature (°C)	24.8	24.9	25.0	25.0	24.8	24.8	24.9	24.9
1050 mg KCl/L	pH (S.U.)	7.74	7.45	7.67	7.35	7.79	7.42	7.72	7.55
	Dissolved oxygen (mg/L)	8.0	7.7	8.0	6.9	8.0	7.8	8.0	7.5
	Conductivity (µmhos/cm)	2040		2040		2090		2050	
	Temperature (°C)	24.9	24.9	24.9	24.8	24.8	24.8	24.9	24.8
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	03-02-21	0.6628	-0.1786	-0.1615	0.0229	0.6895	0.6205	0.7662	0.5894	0.8008	0.4275	0.9515
2	04-06-21	0.7006	-0.1545	-0.1603	0.0226	0.6914	0.6230	0.7672	0.5925	0.8011	0.4287	0.9541
3	05-04-21	0.7509	-0.1244	-0.1589	0.0240	0.6937	0.6212	0.7746	0.5892	0.8103	0.4301	0.9573
4	06-08-21	0.6512	-0.1863	-0.1625	0.0222	0.6879	0.6210	0.7620	0.5907	0.7956	0.4265	0.9493
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	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

Note:

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

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

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

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

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

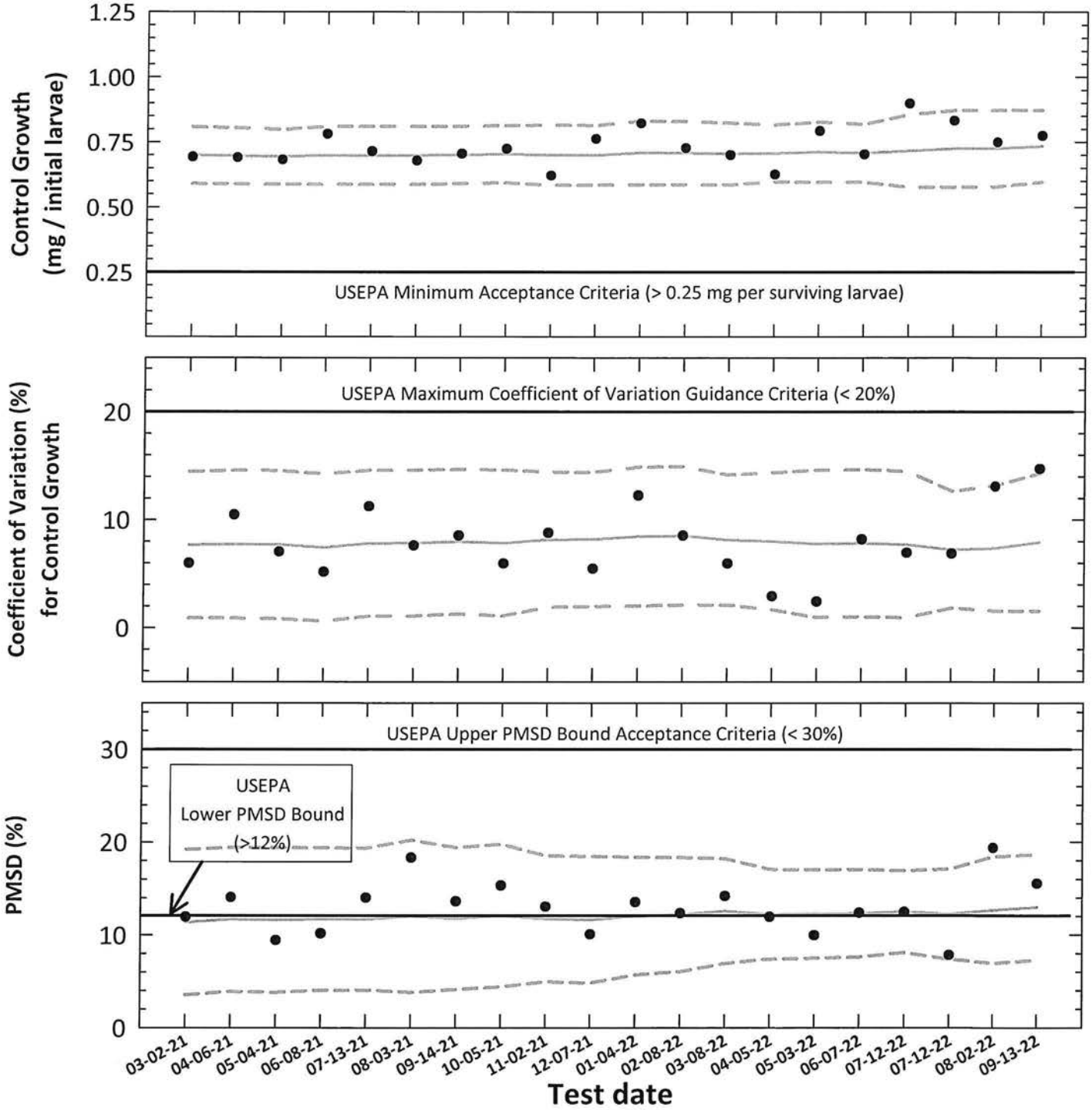
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

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

- - - - **95% Confidence Interval** (mean Control Growth, CV or PMSD ± 2 Standard Deviations)

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival	Control Growth		Test										
			Mean	CV	MSD	PMSD	CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
		(%)	(mg/initial larvae)	(%)		(%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	03-02-21	100	0.693	6.0	0.0827	11.9	0.699	0.590	0.809	7.7	0.9	14.5	11.4	3.5	19.2
2	04-06-21	100	0.691	10.5	0.0970	14.0	0.697	0.589	0.805	7.7	0.9	14.6	11.7	3.9	19.4
3	05-04-21	100	0.682	7.1	0.0643	9.4	0.694	0.589	0.799	7.7	0.8	14.6	11.6	3.8	19.4
4	06-08-21	100	0.781	5.2	0.0794	10.2	0.699	0.587	0.811	7.4	0.6	14.3	11.7	4.0	19.4
5	07-13-21	100	0.715	11.2	0.1000	14.0	0.699	0.587	0.810	7.8	1.1	14.6	11.7	4.0	19.3
6	08-03-21	100	0.678	7.6	0.1243	18.3	0.699	0.588	0.810	7.8	1.1	14.6	12.0	3.8	20.2
7	09-14-21	100	0.706	8.6	0.0960	13.6	0.701	0.591	0.811	8.0	1.3	14.7	11.7	4.1	19.4
8	10-05-21	100	0.724	6.0	0.1109	15.3	0.704	0.594	0.813	7.9	1.1	14.6	12.1	4.4	19.7
9	11-02-21	100	0.621	8.8	0.0810	13.0	0.700	0.585	0.816	8.2	1.9	14.4	11.7	4.9	18.5
10	12-07-21	100	0.763	5.5	0.0767	10.1	0.700	0.585	0.815	8.2	2.0	14.4	11.6	4.8	18.5
11	01-04-22	100	0.822	12.3	0.1112	13.5	0.709	0.587	0.832	8.5	2.0	14.9	12.0	5.7	18.3
12	02-08-22	100	0.728	8.5	0.0898	12.3	0.708	0.587	0.830	8.5	2.1	14.9	12.2	6.0	18.3
13	03-08-22	100	0.701	6.0	0.0994	14.2	0.705	0.587	0.824	8.1	2.1	14.2	12.6	6.9	18.2
14	04-05-22	100	0.626	2.9	0.0747	11.9	0.707	0.598	0.817	8.0	1.6	14.4	12.2	7.4	17.0
15	05-03-22	100	0.793	2.4	0.0790	10.0	0.712	0.596	0.828	7.8	0.9	14.6	12.3	7.5	17.0
16	06-07-22	100	0.704	8.2	0.0871	12.4	0.708	0.597	0.819	7.8	1.0	14.7	12.3	7.6	17.0
17	07-12-22	100	0.899	7.0	0.1121	12.5	0.717	0.577	0.857	7.7	0.9	14.5	12.5	8.1	16.9
18	07-12-22	100	0.833	6.9	0.0653	7.8	0.725	0.578	0.872	7.2	1.8	12.7	12.2	7.4	17.1
19	08-02-22	100	0.750	13.1	0.1452	19.4	0.726	0.578	0.873	7.4	1.5	13.2	12.7	6.9	18.4
20	09-13-22	100	0.774	14.7	0.1203	15.5	0.734	0.596	0.872	7.9	1.5	14.3	13.0	7.3	18.7

Note:

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

Control Mean Growth = USEPA minimum test acceptability criteria ≥ 0.25 mg/surviving larvae.

CV = Coefficient of variation for control growth.
USEPA maximum CV guidance criteria (90th percentile) < 20%

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 12%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 30%.

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: 94

Dilution preparation information:							*Comments:*
KCl Stock INSS number:			INSS 2128				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		*Test information:*	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	09.05.22	*Artemia* CHM number:	CHM1149
Hatch dates and times:	09.12.22 1455 TO 09.13.22 0500	*Drying information for weight determination:*	
Transfer vessel information:	pH = 7.80 S.U. Temperature = 24.0 °C	Date / Time in oven:	09.20.22 0650
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	09.21.22 0650
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	09-13-22	0505 ~~0500~~ 09.13.22	JL	1200	JL	0708	JL	09-08-22 B
1	09-14-22	0500	JL	1100	JL	0700	JL	↓
2	09-15-22	0500	JL	1100	JL	0700	JL	09-08-22 D
3	09-16-22	0500	JL	1100	JL	0700	JL	↓
4	09-17-22	0600	JL	1215	JL	0800	JL	09.14.22 A
5	09-18-22	0600	JL	1200	JL	0800	JL	↓
6	09-19-22	0500	JL	1100	JL	0700	JL	09.15.22 A
7	09-20-22					0608	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	13066468S

Control information:		Acceptance criteria	*Summary of test endpoints:*	
% Mortality:	0%	≤ 20%	7-day LC_{50} (mg/L KCl)	818.5
Average weight per initial larvae:	0.774		NOEC (mg/L KCl)	450
Average weight per surviving larvae:	0.774	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	600
			ChV (mg/L KCl)	519.6
			IC_{25} (mg/L KCl)	668.2

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: 94

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: Turquoise Analyst: EC Date: 08-16-22	13.99	14.74	15.16	12.13	15.96	15.87	15.82	14.00	14.85	14.78	14.94	14.54
*B = Pan + Larvae weight (mg) Analyst: EC Date: 09-22-22	20.64	21.70	24.25	20.40	21.71	24.26	22.87	21.05	21.69	21.34	22.55	21.29
C = Larvae weight (mg) = B – A Analyst: JI	6.65	6.96	9.09	8.27	7.75	8.39	7.05	7.05	6.84	6.56	7.61	6.75
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JI	0.665	0.696	0.909	0.827	0.775	0.839	0.705	0.705	0.684	0.656	0.761	0.675
Average weight per initial number of larvae (mg)	0.774				0.756				0.694			
Percent reduction from control (%)					2.4%				10.4%			

*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:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **94**

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	9 1d	9 1d	9 1d	8 2d	6 4d	6 4d	6 4d
2	10	10	10	10	9	8 1d	9	9	6 2d	5 1d	5 1d	5 1d
3	10	10	10	10	9	8	8 1d	9	3 3d	3 2d	4 1d	4 1d
4	10	10	10	10	7 2d	8 1d	7 1d	8 1d	3	3	3 1d	4
5	10	10	10	10	7	8	7	8	3	3	3	3 1d
6	10	10	10	10	7	8	7	7 1d	2 1d	3	3	3
7	10	10	10	10	7	8	7	7	2	3	3	3
*A = Pan weight (mg) Tray color code: Turquoise Analyst: EC Date: 08-10-22	15.910	14.94	16.03	14.66	15.15	14.58	14.22	14.74	14.89	14.82	15.05	15.00
*B = Pan + Larvae weight (mg) Analyst: EC Date: 09-22-22	22.41	20.91	22.37	21.88	20.53	18.97	19.68	19.42	16.65	17.70	16.80	16.60
C = Larvae weight (mg) = B – A Analyst: JL	6.45	5.97	6.34	7.23	5.38	4.39	5.46	4.68	1.76	2.88	1.75	1.60
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.645	0.597	0.634	0.723	0.538	0.439	0.546	0.468	0.176	0.288	0.175	0.160
Average weight per initial number of larvae (mg)	0.650				0.498				0.200			
Percent reduction from control (%)			16.1%				35.7%				74.2%	

*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:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **94**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 2d	3 2d	5 5d	3 2d
2	2 1d	2 1d	3 2d	3
3	2	2	2 1d	2 1d
4	1	1	0	0
5	0 1d	1	0	0
6	0	0 1d	0	0
7	0	0	0	0
*A = Pan weight (mg) Tray color code: Turquise Analyst: EC Date: 08-16-22	14.66	14.55	14.88	12.88
*B = Pan + Larvae weight (mg) Analyst: ____ Date: ____				
C = Larvae weight (mg) = B – A Analyst: ____				JF 09-19-22
Weight per initial number of larvae (mg) = C / Initial number of larvae JF 09-19-22 Analyst: ____	0	0	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0		100%	

*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:

Environmental Testing Solutions, Inc.

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: 94

Test dates: September 13-20, 2022

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	13.99	20.64	6.65	0.665	0.774	14.7	0.665	100.0	0.774	14.7	Not applicable
	B	10	10	14.74	21.70	6.96	0.696			0.696				
	C	10	10	15.16	24.25	9.09	0.909			0.909				
	D	10	10	12.13	20.40	8.27	0.827			0.827				
300	E	10	10	13.96	21.71	7.75	0.775	0.756	8.5	0.775	100.0	0.756	8.5	2.4
	F	10	10	15.87	24.26	8.39	0.839			0.839				
	G	10	10	15.82	22.87	7.05	0.705			0.705				
	H	10	10	14.00	21.05	7.05	0.705			0.705				
450	I	10	10	14.85	21.69	6.84	0.684	0.694	6.7	0.684	100.0	0.694	6.7	10.4
	J	10	10	14.78	21.34	6.56	0.656			0.656				
	K	10	10	14.94	22.55	7.61	0.761			0.761				
	L	10	10	14.54	21.29	6.75	0.675			0.675				
600	M	10	10	15.96	22.41	6.45	0.645	0.650	8.2	0.645	100.0	0.650	8.2	16.1
	N	10	10	14.94	20.91	5.97	0.597			0.597				
	O	10	10	16.03	22.37	6.34	0.634			0.634				
	P	10	10	14.65	21.88	7.23	0.723			0.723				
750	Q	10	7	15.15	20.53	5.38	0.769	0.691	15.5	0.538	72.5	0.498	10.6	35.7
	R	10	8	14.58	18.97	4.39	0.549			0.439				
	S	10	7	14.22	19.68	5.46	0.780			0.546				
	T	10	7	14.74	19.42	4.68	0.669			0.468				
900	U	10	2	14.89	16.65	1.76	0.880	0.739	28.7	0.176	27.5	0.200	29.7	74.2
	V	10	3	14.82	17.70	2.88	0.960			0.288				
	W	10	3	15.05	16.80	1.75	0.583			0.175				
	X	10	3	15.00	16.60	1.60	0.533			0.160				
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: 0.1203 MSD = Minimum Significant Difference

PMSD: 15.5 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	9/13/2022	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	9/20/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	1.0000
750	0.7000	0.8000	0.7000	0.7000
900	0.2000	0.3000	0.3000	0.3000
1050	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*750	0.7250	0.7250	1.0202	0.9912	1.1071	5.685	4	10.00	10.00	11	40
*900	0.2750	0.2750	0.5506	0.4636	0.5796	10.532	4	10.00	10.00	29	40
1050	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

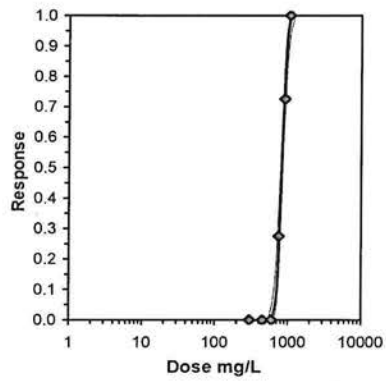
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.75445	0.884	-2E-15	5.27706
Equality of variance cannot be confirmed				

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

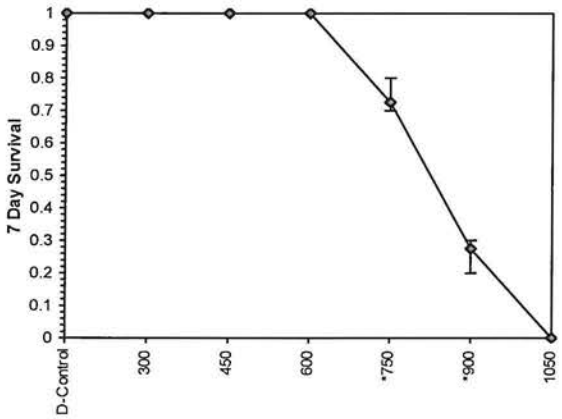
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	19.179	2.61459	14.0544	24.3036	0	2.22464	9.48773	0.69452	2.91303	0.05214	5
Intercept	-50.869	7.63518	-65.834	-35.904							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	619.054	552.053	663.439
EC05	3.355	671.834	615.778	709.395
EC10	3.718	701.786	652.224	735.721
EC15	3.964	722.746	677.691	754.405
EC20	4.158	739.849	698.352	769.909
EC25	4.326	754.844	716.3	783.767
EC40	4.747	793.991	761.834	821.704
EC50	5.000	818.513	788.849	847.29
EC60	5.253	843.791	815.123	875.494
EC75	5.674	887.551	856.913	928.61
EC80	5.842	905.54	872.994	951.76
EC85	6.036	926.969	891.54	980.111
EC90	6.282	954.654	914.75	1017.75
EC95	6.645	997.215	949.269	1077.34
EC99	7.326	1082.24	1015.62	1200.99



Dose-Response Plot



Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	9/13/2022	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	9/20/2022	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	0.6650	0.6960	0.9090	0.8270
300	0.7750	0.8390	0.7050	0.7050
450	0.6840	0.6570	0.7610	0.6750
600	0.6450	0.5970	0.6340	0.7230
750	0.5380	0.4390	0.5460	0.4680
900	0.1760	0.2880	0.1750	0.1600
1050	0.0000	0.0000	0.0000	0.0000

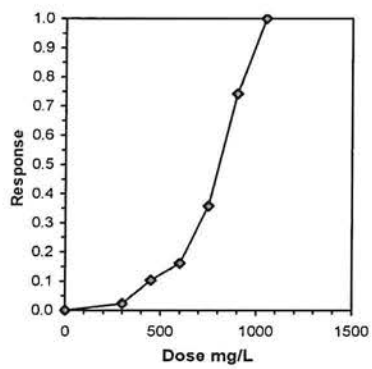
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7743	1.0000	0.7743	0.6650	0.9090	14.726	4				0.7743	1.0000
300	0.7560	0.9764	0.7560	0.7050	0.8390	8.522	4	0.348	2.290	0.1203	0.7560	0.9764
450	0.6943	0.8967	0.6943	0.6570	0.7610	6.611	4	1.523	2.290	0.1203	0.6943	0.8967
*600	0.6498	0.8392	0.6498	0.5970	0.7230	8.153	4	2.371	2.290	0.1203	0.6498	0.8392
750	0.4978	0.6429	0.4978	0.4390	0.5460	10.558	4				0.4978	0.6429
900	0.1998	0.2580	0.1998	0.1600	0.2880	29.680	4				0.1998	0.2580
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)	0.96438	0.844	0.41681	-0.449
Bartlett's Test indicates equal variances (p = 0.42)	2.80653	11.3449		

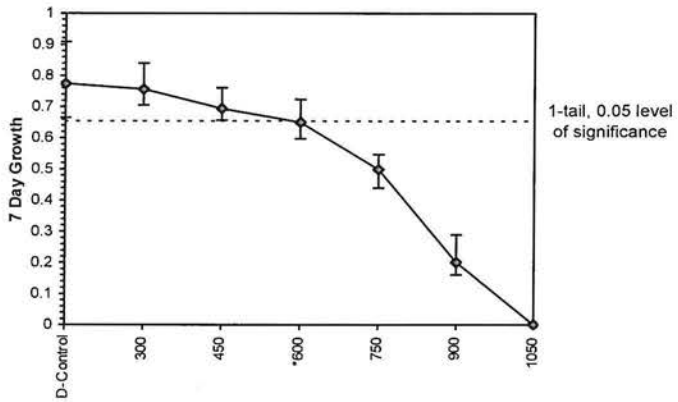
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	450	600	519.615		0.12026	0.15532	0.01311	0.00552	0.12116	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	349.71	122.66	0.00	613.44	-0.1250
IC10	443.74	115.46	59.25	720.34	-0.0575
IC15	571.81	97.63	149.67	698.42	-0.5551
IC20	629.95	63.68	330.83	721.83	-1.2571
IC25	668.15	40.79	482.58	751.67	-0.8510
IC40	766.71	19.07	680.22	803.67	-0.9037
IC50	805.68	13.04	757.54	835.34	-0.2620



Dose-Response Plot



Entered and Reviewed by Jim Summer

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: 94

Daily Chemistry:

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

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	EC	EC N	EC N	EC N	EC N	EC N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.20	7.24	7.22	7.20	7.15	7.29
	Dissolved oxygen (mg/L)	7.9	7.8	7.7	7.6	7.8	7.7
	Conductivity (µmhos/cm)	309		323		317	
	Alkalinity (mg $CaCO_3$/L)	61				61	
	Hardness (mg $CaCO_3$/L)	87				85	
	Temperature (°C)	24.8	24.5	24.8	24.7	24.7	24.7
300 mg KCl/L	pH (S.U.)	7.62	7.36	7.41	7.31	7.47	7.31
	Dissolved oxygen (mg/L)	~~8.34~~ 7.9	7.8	7.6	7.5	7.9	7.7
	Conductivity (µmhos/cm)	853		843		891	
	Temperature (°C)	24.9	24.7	24.8	24.5	24.8	24.7
450 mg KCl/L	pH (S.U.)	7.64	7.41	7.47	7.36	7.49	7.36
	Dissolved oxygen (mg/L)	~~8.34~~ 7.9	7.8	7.6	7.5	7.9	7.7
	Conductivity (µmhos/cm)	1110		1130		1110	
	Temperature (°C)	24.8	24.7	24.8	24.8	24.8	24.5
600 mg KCl/L	pH (S.U.)	7.65	7.42	7.50	7.35	7.52	7.38
	Dissolved oxygen (mg/L)	~~8.44~~ 7.9	7.8	7.4	7.5	8.0	7.8
	Conductivity (µmhos/cm)	1350		1380		1360	
	Temperature (°C)	24.8	24.8	24.9	24.8	24.7	24.7
750 mg KCl/L	pH (S.U.)	7.66	7.46	7.53	7.36	7.55	7.39
	Dissolved oxygen (mg/L)	~~8.5~~ 7.9	7.8	7.4	7.5	8.0	7.8
	Conductivity (µmhos/cm)	1600		1660		1620	
	Temperature (°C)	24.8	24.6	24.9	24.6	24.9	24.6
900 mg KCl/L	pH (S.U.)	7.66	7.47	7.54	7.39	7.57	7.39
	Dissolved oxygen (mg/L)	~~8.6~~ 8.0	7.8	7.6	7.6	8.0	7.8
	Conductivity (µmhos/cm)	1850		1910		1870	
	Temperature (°C)	24.9	24.8	24.9	24.6	24.7	24.6
1050 mg KCl/L	pH (S.U.)	~~7.71~~ (7.67)	7.50	7.58	7.39	7.59	7.46
	Dissolved oxygen (mg/L)	~~8.7~~ 8.0	7.8	7.7	7.7	8.1	7.8
	Conductivity (µmhos/cm)	2120		2170		2150	
	Temperature (°C)	24.9	24.6	24.9	24.6	24.9	24.6
		Initial	Final	Initial	Final	Initial	Final

* REANALYZED + CONFIRMED D.O. N

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: 94

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	EC N	BSL	BSL	BSL	BSL	EC	EC	EC
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.19	7.22	7.28	7.34	7.43	7.29	7.35	7.34
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.6	7.7	7.3	7.8	7.2
	Conductivity (µmhos/cm)	302		310		296		313	
	Alkalinity (mg $CaCO_3$/L)			60				63	
	Hardness (mg $CaCO_3$/L)		09.11.22	87			09.12.22	87	
	Temperature (°C)	24.7	24.5	24.8	24.8	24.8	24.6	24.7	24.6
300 mg KCl/L	pH (S.U.)	7.63	7.33	7.57	7.35	7.74	7.41	7.65	7.40
	Dissolved oxygen (mg/L)	7.8	7.7	7.8	7.6	8.0	7.3	7.8	7.3
	Conductivity (µmhos/cm)	832		847		850		878	
	Temperature (°C)	24.8	24.7	24.8	24.5	24.9	24.5	24.7	24.6
450 mg KCl/L	pH (S.U.)	7.64	7.40	7.60	7.38	7.75	7.47	7.67	7.41
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	7.5	8.0	7.2	7.8	7.2
	Conductivity (µmhos/cm)	1050		1080		1090		1120	
	Temperature (°C)	24.7	24.6	24.8	24.7	24.9	24.5	24.6	24.5
600 mg KCl/L	pH (S.U.)	7.65	7.42	7.63	7.39	7.77	7.48	7.70	7.46
	Dissolved oxygen (mg/L)	7.8	7.6	7.9	7.4	8.0	7.2	7.9	7.2
	Conductivity (µmhos/cm)	1310		1340		1330		1390	
	Temperature (°C)	24.7	24.6	24.8	24.7	24.9	24.7	24.6	24.5
750 mg KCl/L	pH (S.U.)	7.66	7.42	7.64	7.34	7.77	7.54	7.72	7.45
	Dissolved oxygen (mg/L)	7.9	7.6	7.9	7.4	8.0	7.2	7.9	7.2
	Conductivity (µmhos/cm)	1540		1580		1610		1660	
	Temperature (°C)	24.9	24.6	24.7	24.7	25.0	24.7	24.6	24.5
900 mg KCl/L	pH (S.U.)	7.67	7.44	7.65	7.32	7.78	7.57	7.72	7.50
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.4	8.0	7.2	7.8	7.2
	Conductivity (µmhos/cm)	1800		1820		1840		1920	
	Temperature (°C)	24.9	24.5	24.8	24.7	24.8	24.7	24.6	24.7
1050 mg KCl/L	pH (S.U.)	7.69	7.49	7.67	7.42	7.78	7.57	7.74	
	Dissolved oxygen (mg/L)	8.0	7.7	8.0	7.4	8.0	7.1	7.9	
	Conductivity (µmhos/cm)	2070		2110		2120		2190	
	Temperature (°C)	24.9	24.7	24.7	24.6	24.8	24.7	24.7	09.19.22
		Initial	Final	Initial	Final	Initial	Final	Initial	Final