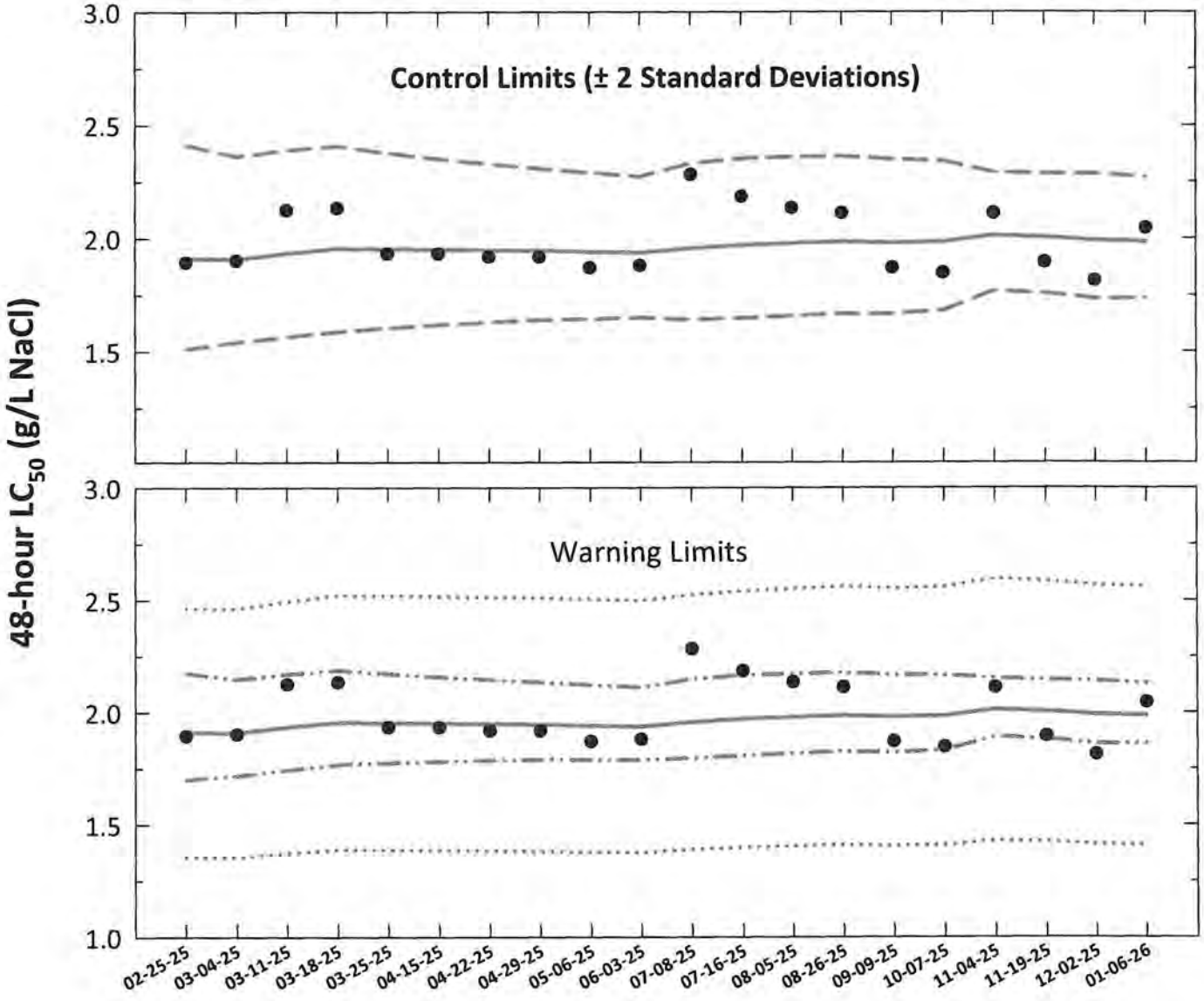
ETS
Environmental Testing Solutions, Inc.

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 $LC_{50} \pm S_{A.75}$ 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.

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	02-25-25	1.8940	0.2774	0.2810	0.0508	1.9097	1.5115	2.4128	1.7012	2.1732	1.3559	2.4635
2	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.3551	2.4621
3	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.3734	2.4953
4	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.3885	2.5227
5	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.3868	2.5197
6	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.3855	2.5173
7	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.3835	2.5137
8	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.3819	2.5107
9	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.3779	2.5036
10	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.3750	2.4982
11	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.3892	2.5240
12	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.3982	2.5404
13	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.4045	2.5517
14	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.4093	2.5605
15	09-09-25	1.8700	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.4051	2.5529
16	10-07-25	1.8463	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.4086	2.5593
17	11-04-25	2.1111	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.4289	2.5962
18	11-19-25	1.8940	0.2774	0.3018	0.0286	2.0035	1.7561	2.2858	1.8800	2.1444	1.4225	2.5845
19	12-02-25	1.8126	0.2583	0.2989	0.0300	1.9902	1.7335	2.2848	1.8612	2.1382	1.4130	2.5673
20	01-06-26	2.0449	0.3107	0.2979	0.0292	1.9856	1.7359	2.2711	1.8598	2.1294	1.4097	2.5614

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.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.29).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETC

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 # 27

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
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 stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2447

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.84	7.88	7.83
	Dissolved oxygen (mg/L)	8.5	8.2	8.1
	Conductivity (µmhos/cm)	289		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	90		
	Temperature (°C)	25.2	25.3	25.0
1000 mg/L	pH (S.U.)	7.97	7.92	7.88
	Dissolved oxygen (mg/L)	8.4	8.2	8.2
	Conductivity (µmhos/cm)	1940		
	Temperature (°C)	25.3	25.2	25.3
1500 mg/L	pH (S.U.)	7.97	7.91	7.89
	Dissolved oxygen (mg/L)	8.4	8.2	8.2
	Conductivity (µmhos/cm)	2760		
	Temperature (°C)	24.9	25.2	24.9
2000 mg/L	pH (S.U.)	7.97	7.93	7.88
	Dissolved oxygen (mg/L)	8.4	8.2	8.2
	Conductivity (µmhos/cm)	3570		
	Temperature (°C)	24.7	25.0	24.9
2500 mg/L	pH (S.U.)	7.96	7.93	7.88
	Dissolved oxygen (mg/L)	8.4	8.2	8.2
	Conductivity (µmhos/cm)	4360		
	Temperature (°C)	24.7	24.9	25.3
3000 mg/L	pH (S.U.)	7.96	7.92	7.89
	Dissolved oxygen (mg/L)	8.4	8.2	8.2
	Conductivity (µmhos/cm)	5230		
	Temperature (°C)	25.0	25.3	25.0

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurem 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 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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN2501000503
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1306647c

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 # 27

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	01.06.26	*0630	JP	0923	JP	2B2	Block	12.30.25A
24	01.07.27			0932	JP			
48 Termination	01.08.27			0927	JP			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	12-30-25 D
Age:	< 24-hours old
Date and time organisms were born between:	01-05-26 1500 TO 01-06-26 0630
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.10
	Temperature (°C): 25.1°C

Survival Data **(number of living organisms):**

Hours	Control				1000 mg/L				1500 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%							

Hours	2000 mg/L				2500 mg/L				3000 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	3^{2d}	3^{2d}	0^{5d}	0^{5d}	3^{2d}	0^{5d}
48 Termination	4^{1d}	0^{5d}	5	4^{1d}	0^{5d}	0^{5d}	0^{3d}	0^{3d}	0	0	0^{3d}	0
Mean Survival	65%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	1936.4
Upper 95% confidence limit (mg NaCl/L)	2159.4
48-hour LC_{50} (mg NaCl/L)	2044.9

Comments:

Test Reviewed by:

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	1/6/2026	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	1/8/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2500	1.0000	1.0000	0.6000	0.6000
3000	0.0000	0.0000	0.6000	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
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	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
2500	0.8000	0.8000	1.1157	0.8861	1.3453	23.763	4	14.00	10.00	4	20
*3000	0.1500	0.1500	0.3907	0.2255	0.8861	84.546	4	10.00	10.00	17	20

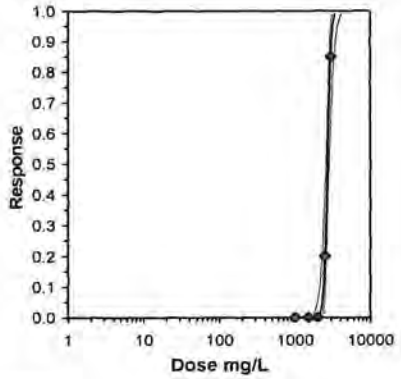
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.74718	0.884	1.43251	4.15572
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2500	3000	2738.61	
Treatments vs D-Control				

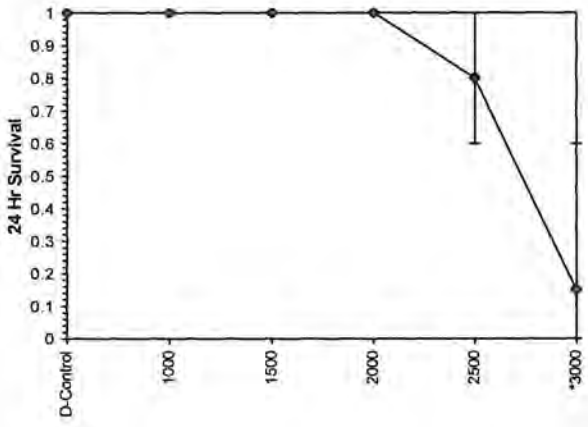
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	23.9605	5.63003	12.9257	34.9954	0	0.0168	7.81472	0.99942	3.43356	0.04174	3
Intercept	-77.27	19.3322	-115.16	-39.379							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	2170.02	1776.92	2349.66
EC05	3.355	2316.9	1999.54	2465.7
EC10	3.718	2399.22	2126.94	2532.83
EC15	3.964	2456.4	2215.6	2581.29
EC20	4.158	2502.82	2287.02	2622.38
EC25	4.326	2543.35	2348.43	2660.09
EC40	4.747	2648.39	2499.09	2770.17
EC50	5.000	2713.66	2582.66	2851.41
EC60	5.253	2780.54	2658.35	2946.84
EC75	5.674	2895.38	2768.28	3135.99
EC80	5.842	2942.26	2808.06	3220.21
EC85	6.036	2997.86	2852.75	3324.03
EC90	6.282	3069.32	2907.32	3462.62
EC95	6.645	3178.37	2986.45	3683.25
EC99	7.326	3393.49	3133.92	4144.72



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	1/6/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	1/8/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.8000	0.0000	1.0000	0.8000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

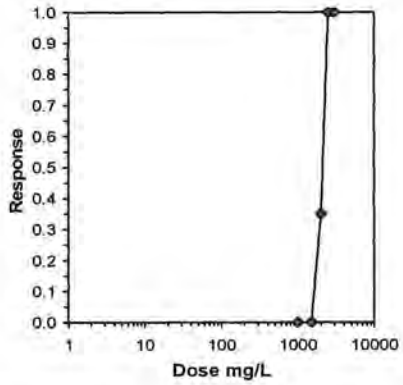
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	0.6500	0.6500	0.9463	0.2255	1.3453	52.146	4	12.00	10.00	7	20
2500	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
3000	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)	0.59568	0.844	-2.1452	8.75084
Equality of variance cannot be confirmed				

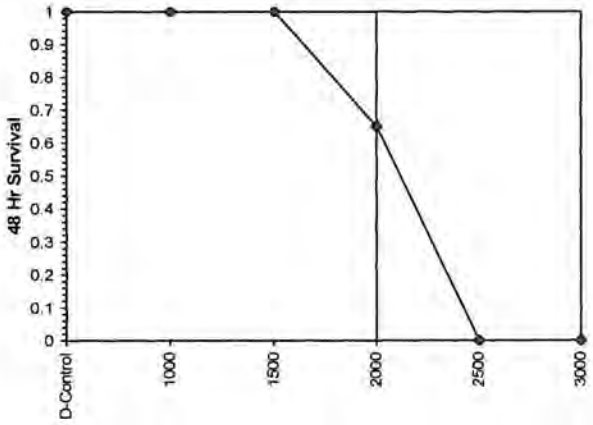
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	2044.85	1936.42	2159.35
5.0%	2055.89	1934.09	2185.37
10.0%	2066.68	1925.94	2217.71
20.0%	2086.87	1881.22	2315.01
Auto-0.0%	2044.85	1936.42	2159.35

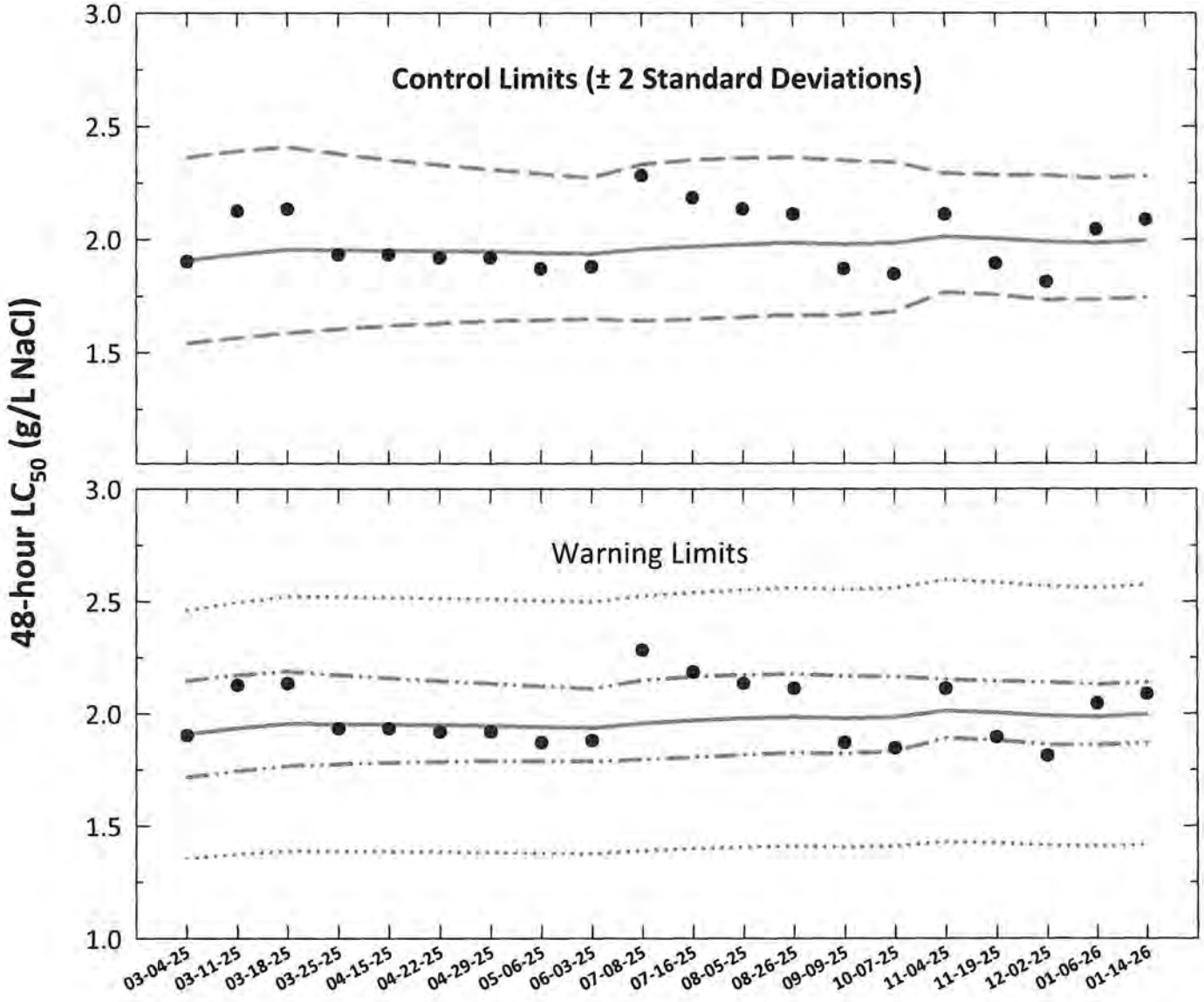


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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 $LC_{50} \pm S_{A.75}$ 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.

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	03-04-25	1.9019	0.2792	0.2807	0.0464	1.9086	1.5417	2.3628	1.7163	2.1466	1.3551	2.4621
2	03-11-25	2.1247	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.3734	2.4953
3	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.3885	2.5227
4	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.3868	2.5197
5	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.3855	2.5173
6	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.3835	2.5137
7	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.3819	2.5107
8	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.3779	2.5036
9	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.3750	2.4982
10	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.3892	2.5240
11	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.3982	2.5404
12	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.4045	2.5517
13	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.4093	2.5605
14	09-09-25	1.8700	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.4051	2.5529
15	10-07-25	1.8463	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.4086	2.5593
16	11-04-25	2.1111	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.4289	2.5962
17	11-19-25	1.8940	0.2774	0.3018	0.0286	2.0035	1.7561	2.2858	1.8800	2.1444	1.4225	2.5845
18	12-02-25	1.8126	0.2583	0.2989	0.0300	1.9902	1.7335	2.2848	1.8612	2.1382	1.4130	2.5673
19	01-06-26	2.0449	0.3107	0.2979	0.0292	1.9856	1.7359	2.2711	1.8598	2.1294	1.4097	2.5614
20	01-14-26	2.0879	0.3197	0.3000	0.0291	1.9953	1.7446	2.2819	1.8697	2.1389	1.4166	2.5739

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.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.29).

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 # 28

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
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 Na stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2451

Chemical Analyses:

Concentration		Hours 0	Hours 24	Hours 48
	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.76	7.83	7.94
	Dissolved oxygen (mg/L)	8.4	8.3	8.2
	Conductivity (µmhos/cm)	294		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.8	25.1	24.9
1000 mg/L	pH (S.U.)	7.99	7.96	7.98
	Dissolved oxygen (mg/L)	8.4	8.3	8.2
	Conductivity (µmhos/cm)	1940		
	Temperature (°C)	24.9	24.8	25.1
1500 mg/L	pH (S.U.)	7.98	7.95	7.99
	Dissolved oxygen (mg/L)	8.4	8.2	8.2
	Conductivity (µmhos/cm)	2740		
	Temperature (°C)	24.9	24.8	24.9
2000 mg/L	pH (S.U.)	7.96	7.97	7.99
	Dissolved oxygen (mg/L)	8.5	8.2	8.3
	Conductivity (µmhos/cm)	3640		
	Temperature (°C)	25.0	25.2	24.9
2500 mg/L	pH (S.U.)	7.95	7.97	7.99
	Dissolved oxygen (mg/L)	8.4	8.2	8.3
	Conductivity (µmhos/cm)	4450		
	Temperature (°C)	24.9	24.7	25.2
3000 mg/L	pH (S.U.)	7.95	7.96	8.00
	Dissolved oxygen (mg/L)	8.4	8.2	8.3
	Conductivity (µmhos/cm)	5330		
	Temperature (°C)	24.9	25.0	25.0

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurement 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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	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 # 28

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	01-14-26	*0400	[illegible]	0741	[illegible]	283	RED	01-08-26A
24	01-15-26			0730	[illegible]			
48 Termination	01-16-26			0731	[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):	01-13-26 C+D
Age:	< 24-hours old
Date and time organisms were born between:	01-13-26 1457 TO 01-14-26 0400
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.09
	Temperature (°C): 25.0

Survival Data **(number of living organisms):**

Hours	Control				1000 mg/L				1500 mg/L			
	Replicate				Replicate				Replicate			
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	S	S	S	S	S	S	S	S	S	S	S	S
48 Termination	S	S	S	S	S	S	S	S	S	S	S	S
Mean Survival	100%				100%				100%			

Hours	2000 mg/L				2500 mg/L				3000 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	S	S	S	S	S	S	S	4 d	2 3d	2 3d	1 4d	1 4d
48 Termination	3 3d	3 3d	3 3d	4 d	0	0	0	1 3d	0 2d	0 2d	0 d	0 d
Mean Survival	65%				5%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	Probit
Lower 95% confidence limit (mg NaCl/L)	1960.6
Upper 95% confidence limit (mg NaCl/L)	2206.9
48-hour LC_{50} (mg NaCl/L)	2097.9

Comments:

Test Reviewed by: [illegible]

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	1/14/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	1/16/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	1.0000	1.0000	1.0000	1.0000
2500	1.0000	1.0000	1.0000	0.8000
3000	0.4000	0.4000	0.2000	0.2000

			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
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	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
2500	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
*3000	0.3000	0.3000	0.5742	0.4636	0.6847	22.229	4	10.00	10.00	14	20

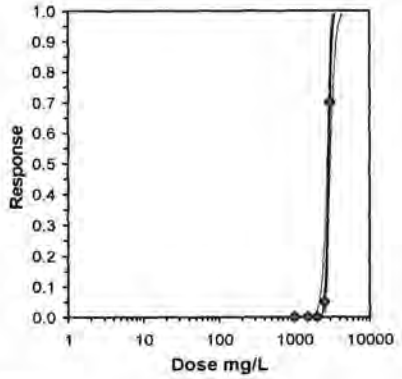
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77039	0.884	-0.9587	2.47243
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2500	3000	2738.61	
Treatments vs D-Control				

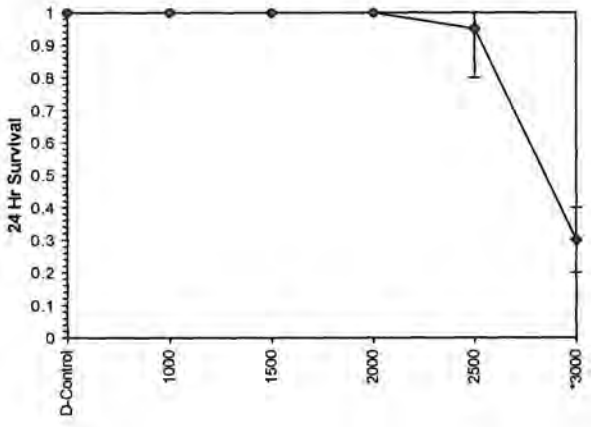
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	27.4019	7.02027	13.6422	41.1616	0	0.00017	7.81472	1	3.45798	0.03649	3
Intercept	-89.755	24.2558	-137.3	-42.214							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	2360.93	1935.43	2536.32
EC05	3.355	2500.08	2164.03	2643.81
EC10	3.718	2577.58	2293.89	2706.32
EC15	3.964	2631.22	2383.69	2751.84
EC20	4.158	2674.65	2455.55	2790.86
EC25	4.326	2712.48	2516.84	2827.11
EC40	4.747	2810.19	2664.13	2935.93
EC50	5.000	2870.65	2743.02	3018.54
EC60	5.253	2932.42	2812.57	3116.36
EC75	5.674	3038.05	2911.54	3309.26
EC80	5.842	3081.02	2947.06	3394.5
EC85	6.036	3131.87	2986.88	3499.13
EC90	6.282	3197.05	3035.4	3638.19
EC95	6.645	3296.16	3105.55	3858.5
EC99	7.326	3490.42	3235.63	4316.29



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	1/14/2026	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	1/16/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.6000	0.6000	0.6000	0.8000
2500	0.0000	0.0000	0.0000	0.2000
3000	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
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.6500	0.6500	0.9413	0.8861	1.1071	11.742	4	10.00	10.00	7	20
*2500	0.0500	0.0500	0.2850	0.2255	0.4636	41.771	4	10.00	10.00	19	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

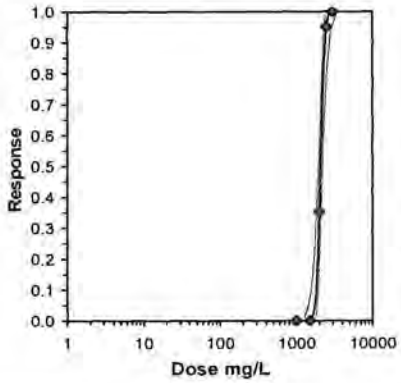
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.65979	0.868	1.98132	4.10888
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

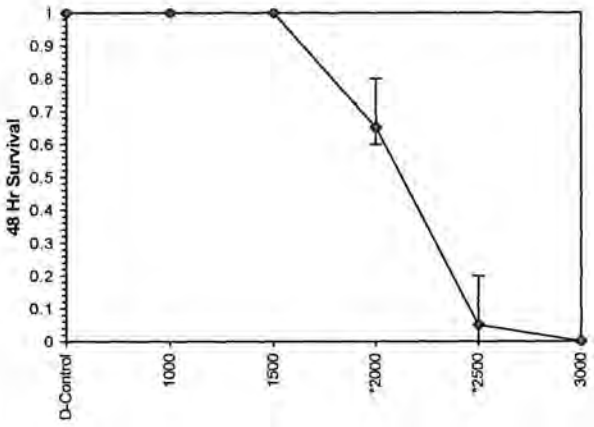
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	21.4313	5.18429	11.2701	31.5925	0	0.03557	7.81472	0.99823	3.31972	0.04666	3
Intercept	-66.146	17.2411	-99.938	-32.353							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1626.17	1269.86	1788.05
EC05	3.355	1749.71	1454.29	1885.92
EC10	3.718	1819.35	1561.49	1942.54
EC15	3.964	1867.9	1636.91	1983.32
EC20	4.158	1907.41	1698.24	2017.77
EC25	4.326	1941.97	1751.45	2049.23
EC40	4.747	2031.86	1884.68	2140.18
EC50	5.000	2087.93	1960.63	2206.94
EC60	5.253	2145.54	2030.44	2286.1
EC75	5.674	2244.85	2131.6	2447.26
EC80	5.842	2285.53	2167.69	2520.63
EC85	6.036	2333.87	2207.85	2612.09
EC90	6.282	2396.15	2256.45	2735.52
EC95	6.645	2491.53	2326.35	2934.45
EC99	7.326	2680.8	2455.81	3357.74

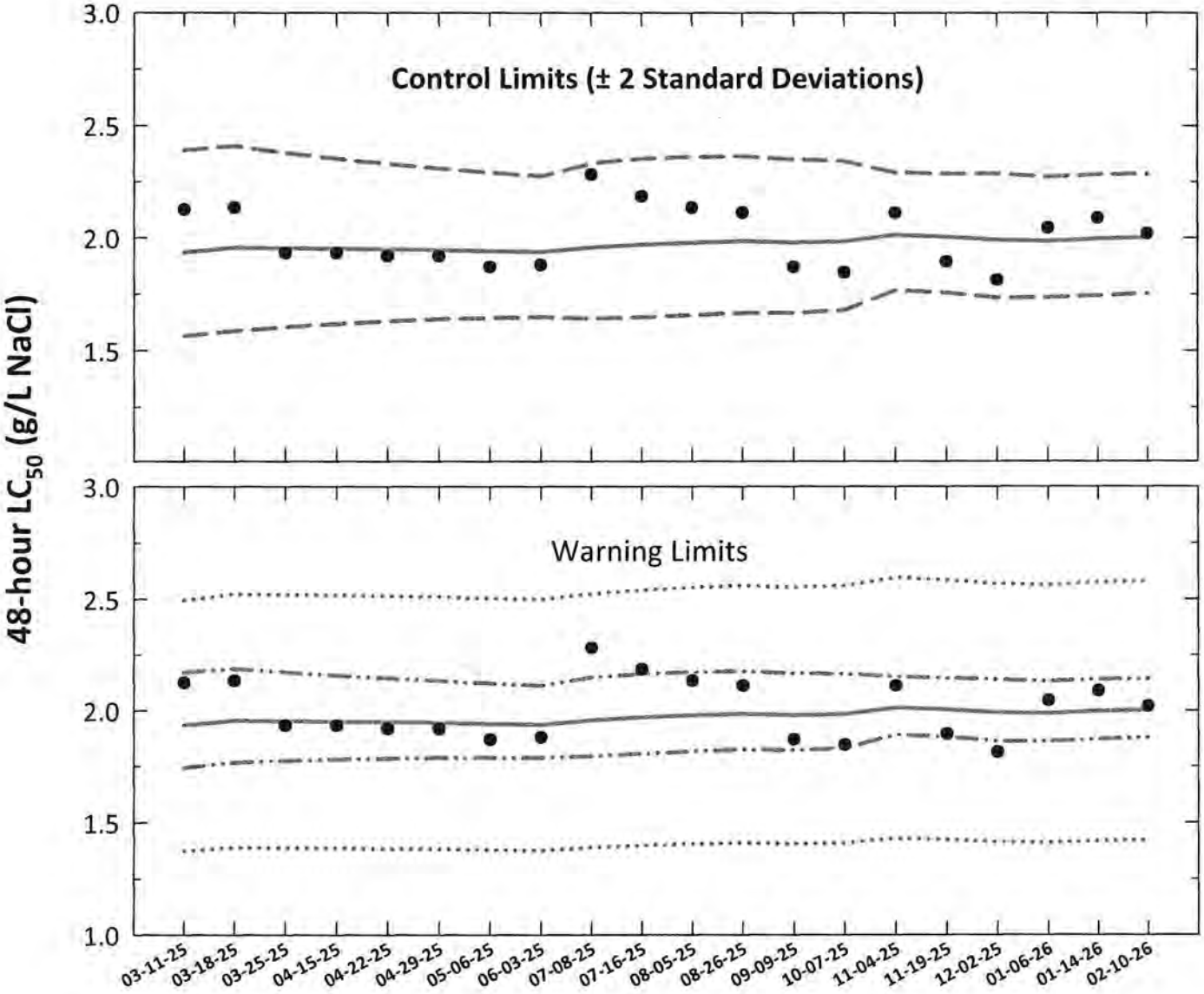


Dose-Response Plot



Environmental Testing Solutions, Inc.

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} ± $S_{A.75}$ 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.

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**03-11-25**	**2.1247**	0.3273	0.2865	0.0460	1.9344	1.5653	2.3905	1.7436	2.1701	1.3734	2.4953
2	**03-18-25**	**2.1340**	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.3885	2.5227
3	**03-25-25**	**1.9328**	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.3868	2.5197
4	**04-15-25**	**1.9328**	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.3855	2.5173
5	**04-22-25**	**1.9184**	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.3835	2.5137
6	**04-29-25**	**1.9184**	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.3819	2.5107
7	**05-06-25**	**1.8700**	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.3779	2.5036
8	**06-03-25**	**1.8799**	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.3750	2.4982
9	**07-08-25**	**2.2819**	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.3892	2.5240
10	**07-16-25**	**2.1842**	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.3982	2.5404
11	**08-05-25**	**2.1340**	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.4045	2.5517
12	**08-26-25**	**2.1117**	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.4093	2.5605
13	**09-09-25**	**1.8700**	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.4051	2.5529
14	**10-07-25**	**1.8463**	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.4086	2.5593
15	**11-04-25**	**2.1111**	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.4289	2.5962
16	**11-19-25**	**1.8940**	0.2774	0.3018	0.0286	2.0035	1.7561	2.2858	1.8800	2.1444	1.4225	2.5845
17	**12-02-25**	**1.8126**	0.2583	0.2989	0.0300	1.9902	1.7335	2.2848	1.8612	2.1382	1.4130	2.5673
18	**01-06-26**	**2.0449**	0.3107	0.2979	0.0292	1.9856	1.7359	2.2711	1.8598	2.1294	1.4097	2.5614
19	**01-14-26**	**2.0879**	0.3197	0.3000	0.0291	1.9953	1.7446	2.2819	1.8697	2.1389	1.4166	2.5739
20	**02-10-26**	**2.0189**	0.3051	0.3013	0.0287	2.0012	1.7531	2.2845	1.8772	2.1428	1.4209	2.5816

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.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.29).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETC

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 # 29

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
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 N stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2451

Chemical Analyses:

		Hours		
Concentration	Analyst	0	24	48
Control, MHSW		XL	XL	XL
	pH (S.U.)	7.82	7.87	7.96
	Dissolved oxygen (mg/L)	8.4	8.2	8.1
	Conductivity (µmhos/cm)	295		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.3	25.2	25.1
1000 mg/L	pH (S.U.)	8.00	7.91	7.98
	Dissolved oxygen (mg/L)	8.5	8.2	8.3
	Conductivity (µmhos/cm)	2050		
	Temperature (°C)	24.5	24.9	25.0
1500 mg/L	pH (S.U.)	8.00	7.91	7.98
	Dissolved oxygen (mg/L)	8.6	8.2	8.3
	Conductivity (µmhos/cm)	2820		
	Temperature (°C)	24.5	24.9	25.2
2000 mg/L	pH (S.U.)	7.99	7.91	7.99
	Dissolved oxygen (mg/L)	8.6	8.2	8.3
	Conductivity (µmhos/cm)	3610		
	Temperature (°C)	24.5	25.1	25.2
2500 mg/L	pH (S.U.)	7.98	7.90	7.99
	Dissolved oxygen (mg/L)	8.6	8.2	8.3
	Conductivity (µmhos/cm)	4460		
	Temperature (°C)	24.6	24.6	24.8
3000 mg/L	pH (S.U.)	7.98	7.90	8.00
	Dissolved oxygen (mg/L)	8.5	8.2	8.3
	Conductivity (µmhos/cm)	5290		
	Temperature (°C)	24.8	25.0	24.8

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurement 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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664705

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 # 29

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	02.10.26	* 0540	JJ	0835	JP	2C3	Rec	02.04.26B
24	02.11.26			0833	JP			
48 Termination	02.12.26			0830	JP			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	02.03.26 D
Age:	< 24-hours old
Date and time organisms were born between:	02.09.26 1411 TO 02.10.26 0540
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.90
	Temperature (°C): 24.4°C

Survival Data **(number of living organisms):**

Hours	Control				1000 mg/L				1500 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	2000 mg/L				2500 mg/L				3000 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	4^{1d}	5	5	5	1^{4d}	3^{2d}	3^{2d}	4^{1d}	0^{5d}	0^{5d}	0^{5d}	1^{4d}
48 Termination	3^{1d}	3^{2d}	2^{3d}	4^{1d}	0^{1d}	0^{2d}	0^{3d}	0^{4d}	0	0	0	0^{1d}
Mean Survival	60%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	1909.0
Upper 95% confidence limit (mg NaCl/L)	2135.1
48-hour LC_{50} (mg NaCl/L)	2018.9

Comments:

Test Reviewed by: JP

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	2/10/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	2/12/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.8000	1.0000	1.0000	1.0000
2500	0.2000	0.6000	0.6000	0.8000
3000	0.0000	0.0000	0.0000	0.2000

			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
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
*2500	0.5500	0.5500	0.8357	0.4636	1.1071	32.195	4	10.00	10.00	9	20
*3000	0.0500	0.0500	0.2850	0.2255	0.4636	41.771	4	10.00	10.00	19	20

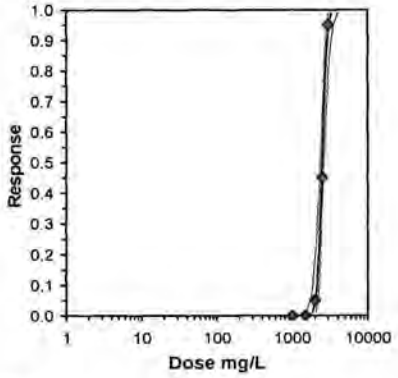
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.79806	0.884	-0.9845	5.30123
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

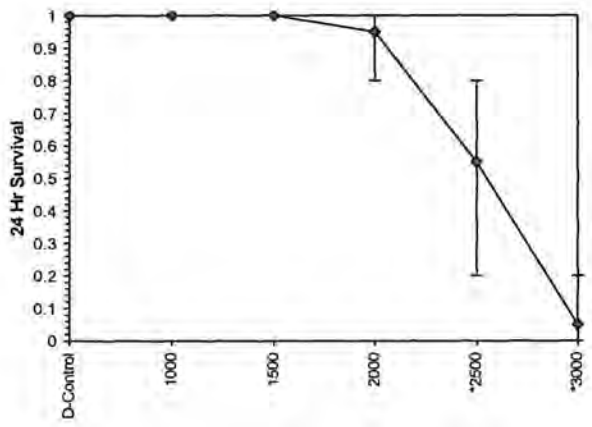
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	18.6599	3.8382	11.137	26.1827	0	0.44793	7.81472	0.93017	3.39821	0.05359	3
Intercept	-58.41	13.0608	-84.009	-32.811							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1877.31	1519.9	2066.54
EC05	3.355	2042.01	1743.24	2202.53
EC10	3.718	2135.64	1873.13	2281.44
EC15	3.964	2201.22	1964.53	2338.24
EC20	4.158	2254.78	2038.87	2386.11
EC25	4.326	2301.76	2103.41	2429.66
EC40	4.747	2424.54	2265.55	2553.86
EC50	5.000	2501.54	2359.1	2642.7
EC60	5.253	2580.98	2446.96	2745.31
EC75	5.674	2718.65	2579.69	2948.16
EC80	5.842	2775.3	2628.82	3039.12
EC85	6.036	2842.83	2684.49	3151.95
EC90	6.282	2930.13	2753.02	3303.72
EC95	6.645	3064.48	2853.3	3547.82
EC99	7.326	3333.33	3042.76	4066.88



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	2/10/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	2/12/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.6000	0.6000	0.4000	0.8000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

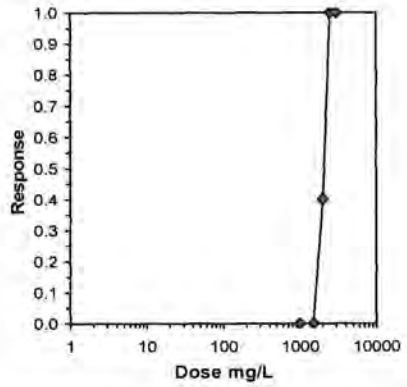
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.6000	0.6000	0.8910	0.6847	1.1071	19.366	4	10.00	10.00	8	20
2500	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
3000	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)	0.52449	0.844	0.21873	7.51217
Equality of variance cannot be confirmed				

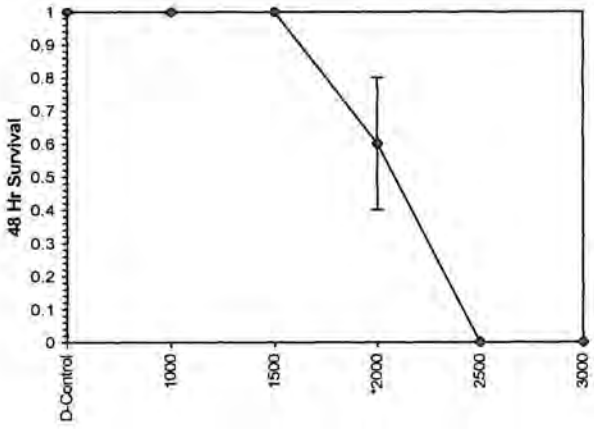
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

Trimmed Spearman-Karber

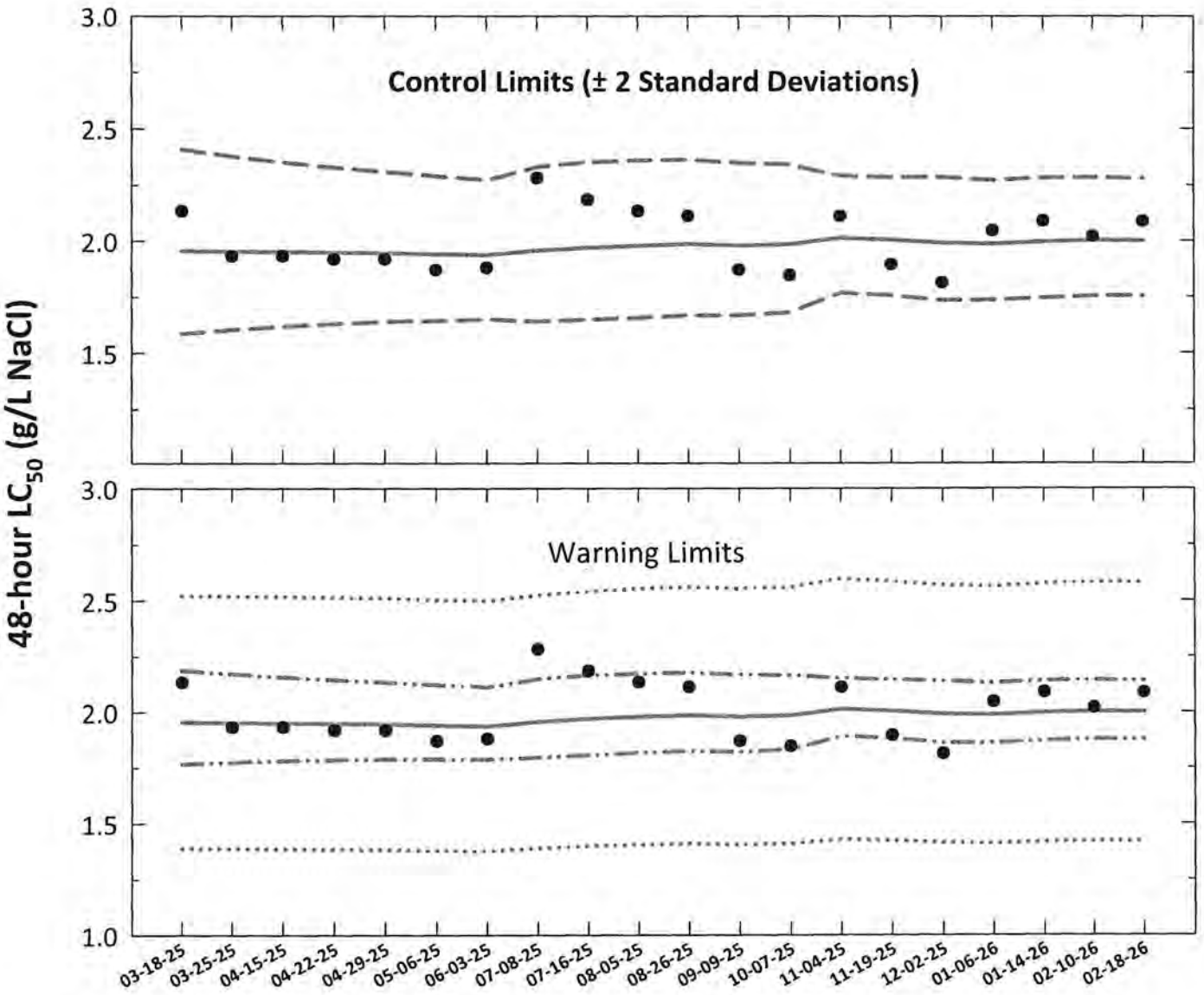
Trim Level	EC50	95% CL	
0.0%	2018.9	1909.03	2135.1
5.0%	2027.29	1904.4	2158.12
10.0%	2035.62	1895.04	2186.63
20.0%	2051.89	1853.16	2271.93
Auto-0.0%	2018.9	1909.03	2135.1



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 $LC_{50} \pm S_{A.75}$ 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.

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	03-18-25	2.1340	0.3292	0.2913	0.0453	1.9556	1.5874	2.4092	1.7673	2.1875	1.3885	2.5227
2	03-25-25	1.9328	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.3868	2.5197
3	04-15-25	1.9328	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.3855	2.5173
4	04-22-25	1.9184	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.3835	2.5137
5	04-29-25	1.9184	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.3819	2.5107
6	05-06-25	1.8700	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.3779	2.5036
7	06-03-25	1.8799	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.3750	2.4982
8	07-08-25	2.2819	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.3892	2.5240
9	07-16-25	2.1842	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.3982	2.5404
10	08-05-25	2.1340	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.4045	2.5517
11	08-26-25	2.1117	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.4093	2.5605
12	09-09-25	1.8700	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.4051	2.5529
13	10-07-25	1.8463	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.4086	2.5593
14	11-04-25	2.1111	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.4289	2.5962
15	11-19-25	1.8940	0.2774	0.3018	0.0286	2.0035	1.7561	2.2858	1.8800	2.1444	1.4225	2.5845
16	12-02-25	1.8126	0.2583	0.2989	0.0300	1.9902	1.7335	2.2848	1.8612	2.1382	1.4130	2.5673
17	01-06-26	2.0449	0.3107	0.2979	0.0292	1.9856	1.7359	2.2711	1.8598	2.1294	1.4097	2.5614
18	01-14-26	2.0879	0.3197	0.3000	0.0291	1.9953	1.7446	2.2819	1.8697	2.1389	1.4166	2.5739
19	02-10-26	2.0189	0.3051	0.3013	0.0287	2.0012	1.7531	2.2845	1.8772	2.1428	1.4209	2.5816
20	02-18-26	2.0862	0.3193	0.3009	0.0284	1.9994	1.7541	2.2790	1.8767	2.1392	1.4196	2.5792

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.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.29).

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 # 70

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
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 N stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2451

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.83	7.81	7.97
	Dissolved oxygen (mg/L)	8.5	8.2	8.2
	Conductivity (µmhos/cm)	294		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	92		
	Temperature (°C)	25.1	25.2	25.2
1000 mg/L	pH (S.U.)	7.88	7.89	7.99
	Dissolved oxygen (mg/L)	8.5	8.2	8.2
	Conductivity (µmhos/cm)	1930		
	Temperature (°C)	24.9	25.0	25.0
1500 mg/L	pH (S.U.)	7.89	7.90	8.00
	Dissolved oxygen (mg/L)	8.5	8.2	8.2
	Conductivity (µmhos/cm)	2760		
	Temperature (°C)	24.9	25.1	25.1
2000 mg/L	pH (S.U.)	7.90	7.91	8.00
	Dissolved oxygen (mg/L)	8.5	8.2	8.1
	Conductivity (µmhos/cm)	3510		
	Temperature (°C)	25.0	25.1	25.1
2500 mg/L	pH (S.U.)	7.91	7.91	8.00
	Dissolved oxygen (mg/L)	8.5	8.2	8.1
	Conductivity (µmhos/cm)	4320		
	Temperature (°C)	25.0	25.1	25.1
3000 mg/L	pH (S.U.)	7.91	7.91	8.00
	Dissolved oxygen (mg/L)	8.5	8.2	8.1
	Conductivity (µmhos/cm)	5170		
	Temperature (°C)	25.0	25.2	24.9

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measuremen 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 th 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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130604681

Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	1/18/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	1/20/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.4000	0.6000	0.6000	0.8000
2500	0.2000	0.2000	0.0000	0.0000
3000	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
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.6000	0.6000	0.8910	0.6847	1.1071	19.366	4	10.00	10.00	8	20
*2500	0.1000	0.1000	0.3446	0.2255	0.4636	39.900	4	10.00	10.00	18	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

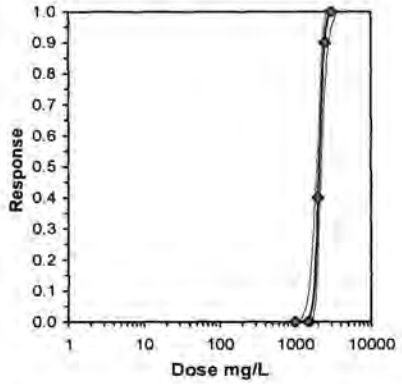
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.78876	0.868	0.11449	2.32768
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

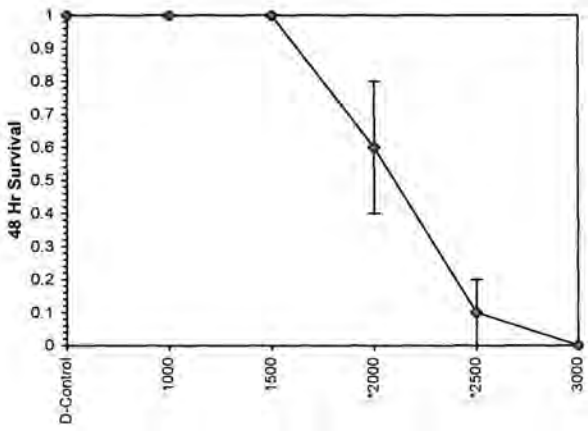
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.6867	3.74573	10.345	25.0283	0	0.312	7.81472	0.95776	3.31935	0.05654	4
Intercept	-53.708	12.4737	-78.157	-29.26							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1541.05	1208.14	1715.89
EC05	3.355	1684.02	1400.52	1834.1
EC10	3.718	1765.58	1513.49	1902.69
EC15	3.964	1822.83	1593.52	1951.99
EC20	4.158	1869.66	1658.99	1993.45
EC25	4.326	1910.78	1716.18	2031.06
EC40	4.747	2018.47	1861.6	2137.64
EC50	5.000	2086.16	1946.97	2213.47
EC60	5.253	2156.11	2027.98	2301.34
EC75	5.674	2277.62	2150.51	2477.65
EC80	5.842	2327.72	2195.49	2557.9
EC85	6.036	2387.52	2246.13	2658.24
EC90	6.282	2464.93	2308.04	2794.31
EC95	6.645	2584.32	2398.01	3015.1
EC99	7.326	2824.09	2566.96	3490.11

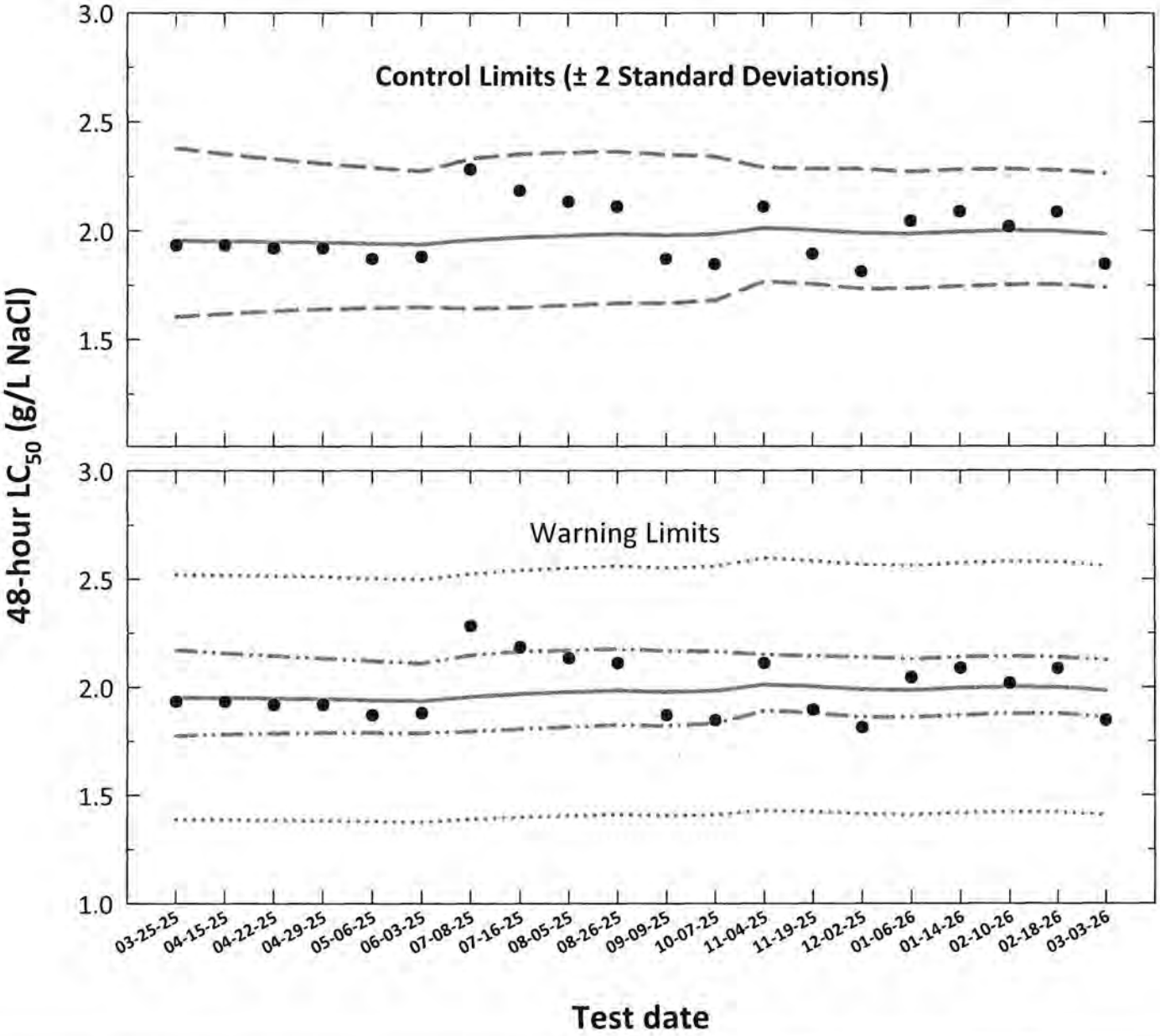


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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



Test date

- ● **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} ± $S_{A.75}$ 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.

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		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**03-25-25**	**1.9328**	0.2862	0.2908	0.0427	1.9533	1.6044	2.3781	1.7746	2.1708	1.3868	2.5197
2	**04-15-25**	**1.9328**	0.2862	0.2904	0.0406	1.9514	1.6189	2.3522	1.7810	2.1568	1.3855	2.5173
3	**04-22-25**	**1.9184**	0.2829	0.2897	0.0387	1.9486	1.6303	2.3292	1.7853	2.1439	1.3835	2.5137
4	**04-29-25**	**1.9184**	0.2829	0.2892	0.0371	1.9463	1.6404	2.3093	1.7891	2.1328	1.3819	2.5107
5	**05-06-25**	**1.8700**	0.2718	0.2880	0.0360	1.9407	1.6444	2.2905	1.7881	2.1209	1.3779	2.5036
6	**06-03-25**	**1.8799**	0.2741	0.2870	0.0349	1.9366	1.6494	2.2738	1.7883	2.1107	1.3750	2.4982
7	**07-08-25**	**2.2819**	0.3583	0.2915	0.0381	1.9566	1.6418	2.3318	1.7957	2.1483	1.3892	2.5240
8	**07-16-25**	**2.1842**	0.3393	0.2943	0.0387	1.9693	1.6481	2.3531	1.8062	2.1642	1.3982	2.5404
9	**08-05-25**	**2.1340**	0.3292	0.2962	0.0384	1.9781	1.6575	2.3607	1.8160	2.1715	1.4045	2.5517
10	**08-26-25**	**2.1117**	0.3246	0.2977	0.0379	1.9849	1.6672	2.3632	1.8248	2.1755	1.4093	2.5605
11	**09-09-25**	**1.8700**	0.2718	0.2964	0.0373	1.9790	1.6665	2.3501	1.8211	2.1665	1.4051	2.5529
12	**10-07-25**	**1.8463**	0.2663	0.2975	0.0360	1.9839	1.6805	2.3421	1.8310	2.1645	1.4086	2.5593
13	**11-04-25**	**2.1111**	0.3245	0.3038	0.0282	2.0126	1.7675	2.2917	1.8908	2.1513	1.4289	2.5962
14	**11-19-25**	**1.8940**	0.2774	0.3018	0.0286	2.0035	1.7561	2.2858	1.8800	2.1444	1.4225	2.5845
15	**12-02-25**	**1.8126**	0.2583	0.2989	0.0300	1.9902	1.7335	2.2848	1.8612	2.1382	1.4130	2.5673
16	**01-06-26**	**2.0449**	0.3107	0.2979	0.0292	1.9856	1.7359	2.2711	1.8598	2.1294	1.4097	2.5614
17	**01-14-26**	**2.0879**	0.3197	0.3000	0.0291	1.9953	1.7446	2.2819	1.8697	2.1389	1.4166	2.5739
18	**02-10-26**	**2.0189**	0.3051	0.3013	0.0287	2.0012	1.7531	2.2845	1.8772	2.1428	1.4209	2.5816
19	**02-18-26**	**2.0862**	0.3193	0.3009	0.0284	1.9994	1.7541	2.2790	1.8767	2.1392	1.4196	2.5792
20	**03-03-26**	**1.8463**	0.2663	0.2978	0.0286	1.9850	1.7400	2.2644	1.8616	2.1258	1.4093	2.5606

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.

$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA. ($S_{A.75}$ = 0.29).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETC

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 # 31

Dilution Preparation:

Test concentrations (mg/L NaCl)	1000	1500	2000	2500	3000
mL Stock solution	2.0	3.0	4.0	5.0	6.0
mL Dilution water (MHSW)	198	197	196	195	194
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 NaC stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2466

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	JN	XL	XL
Control, MHSW	pH (S.U.)	8.16	7.91	7.80
	Dissolved oxygen (mg/L)	8.2	8.3	8.2
	Conductivity (µmhos/cm)	293		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	25.0	25.3	25.3
1000 mg/L	pH (S.U.)	7.93	7.93	7.83
	Dissolved oxygen (mg/L)	8.3	8.3	8.1
	Conductivity (µmhos/cm)	1910		
	Temperature (°C)	24.9	25.0	25.1
1500 mg/L	pH (S.U.)	7.95	7.94	7.85
	Dissolved oxygen (mg/L)	8.3	8.3	8.1
	Conductivity (µmhos/cm)	2720		
	Temperature (°C)	24.4	25.4	24.9
2000 mg/L	pH (S.U.)	7.99	7.94	7.87
	Dissolved oxygen (mg/L)	8.3	8.3	8.1
	Conductivity (µmhos/cm)	3510		
	Temperature (°C)	24.6	25.3	24.9
2500 mg/L	pH (S.U.)	7.99	7.94	7.87
	Dissolved oxygen (mg/L)	8.3	8.4	8.1
	Conductivity (µmhos/cm)	4280		
	Temperature (°C)	24.5	25.1	24.6
3000 mg/L	pH (S.U.)	7.99	7.94	
	Dissolved oxygen (mg/L)	8.3	8.3	
	Conductivity (µmhos/cm)	5010		
	Temperature (°C)	24.8	24.9	

JN 05-06-26

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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664705

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 # 31

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	03-03-26	*0753	JP	1000	JP	264	ORANGE	02-25-26A
24	03-04-26			1021	JP			
48 Termination	03-05-26			1006	JP			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	02-24-26 JAY A
Age:	< 24-hours old
Date and time organisms were born between:	03-02-26 @ 1147 #03-03-26 TO 03-03-26 0753
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.18
	Temperature (°C): 24.1°C

Survival Data **(number of living organisms):**

Hours	Control				1000 mg/L				1500 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 #03-04-26	5	5	5	5	5	5	5	5
48 Termination	5	5	5 #03-04-26 (5)	5	5	5	5	5	5	5	5	5
Mean Survival	100%				100%				100%			

Hours	2000 mg/L				2500 mg/L				3000 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	4 d	5	5	3 d	1 d	1 d	2 d	0 d	0 d	0 d	0 d
48 Termination	1 d	0 d	2 d	2 d	0 d	0 d	0 d	0 d	0	0	0	0
Mean Survival	25%				0%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	SK
Lower 95% confidence limit (mg NaCl/L)	~~835.7~~ (1752.2)
Upper 95% confidence limit (mg NaCl/L)	2056.6 (1939.9)
48-hour LC_{50} (mg NaCl/L)	~~1443.0~~ (1846.3) #03-17-26

Comments:

Test Reviewed by:

Statistical Analyses

Acute Daphnid Test-24 Hr Survival

Start Date:	3/3/2026	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	3/5/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	1.0000	0.8000	1.0000	1.0000
2500	0.6000	0.2000	0.8000	0.4000
3000	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
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	0.9500	0.9500	1.2857	1.1071	1.3453	9.261	4	16.00	10.00	1	20
*2500	0.5000	0.5000	0.7854	0.4636	1.1071	35.048	4	10.00	10.00	10	20
3000	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

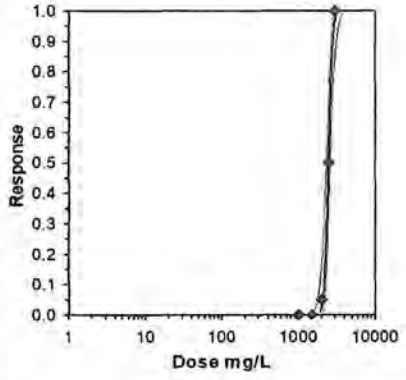
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.77684	0.905	-0.175	4.5884
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2500	2236.07	
Treatments vs D-Control				

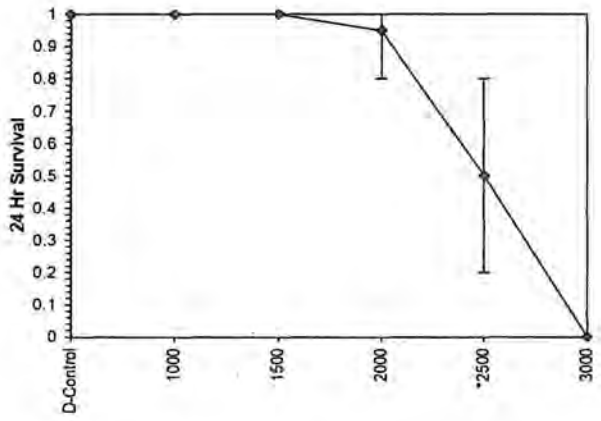
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	21.7796	4.61963	12.7251	30.8341	0	1.41886	7.81472	0.70112	3.3893	0.04591	5
Intercept	-68.818	15.6867	-99.564	-38.072							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1916.39	1576.7	2089.69
EC05	3.355	2059.56	1777.38	2206.51
EC10	3.718	2140.21	1892.56	2273.89
EC15	3.964	2196.39	1973.01	2322.21
EC20	4.158	2242.1	2038.13	2362.8
EC25	4.326	2282.07	2094.47	2399.6
EC40	4.747	2385.97	2235.51	2503.75
EC50	5.000	2450.74	2316.81	2577.51
EC60	5.253	2517.27	2393.18	2662.21
EC75	5.674	2631.88	2507.95	2829.13
EC80	5.842	2678.8	2550.01	2903.92
EC85	6.036	2734.54	2597.32	2996.6
EC90	6.282	2806.33	2655.05	3120.99
EC95	6.645	2916.22	2738.64	3320.19
EC99	7.326	3134.08	2894.31	3739.46



Dose-Response Plot



Statistical Analyses

Acute Daphnid Test-48 Hr Survival

Start Date:	3/3/2026	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/5/2026	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
1000	1.0000	1.0000	1.0000	1.0000
1500	1.0000	1.0000	1.0000	1.0000
2000	0.2000	0.0000	0.4000	0.4000
2500	0.0000	0.0000	0.0000	0.0000
3000	0.0000	0.0000	0.0000	0.0000

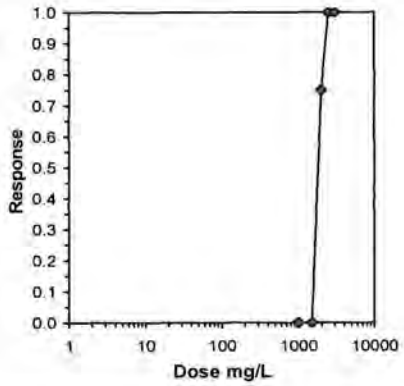
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
1500	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2000	0.2500	0.2500	0.5146	0.2255	0.6847	42.578	4	10.00	10.00	15	20
2500	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20
3000	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)	0.6454	0.844	-1.1712	5.65678
Equality of variance cannot be confirmed				

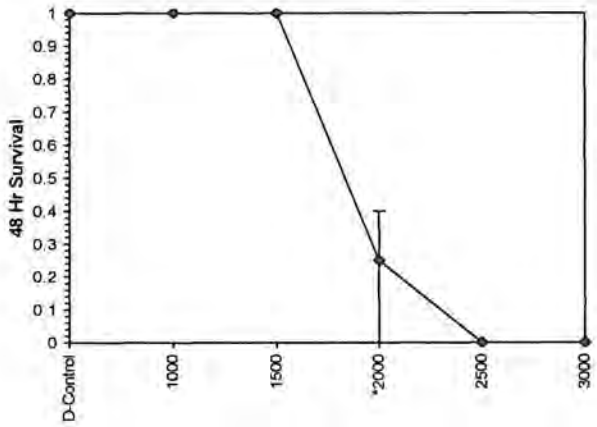
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	2000	1732.05	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	1846.26	1757.16	1939.87
5.0%	1837.79	1740.95	1940.02
10.0%	1830.17	1726.83	1939.7
20.0%	1819.05	1708.51	1936.73
Auto-0.0%	1846.26	1757.16	1939.87

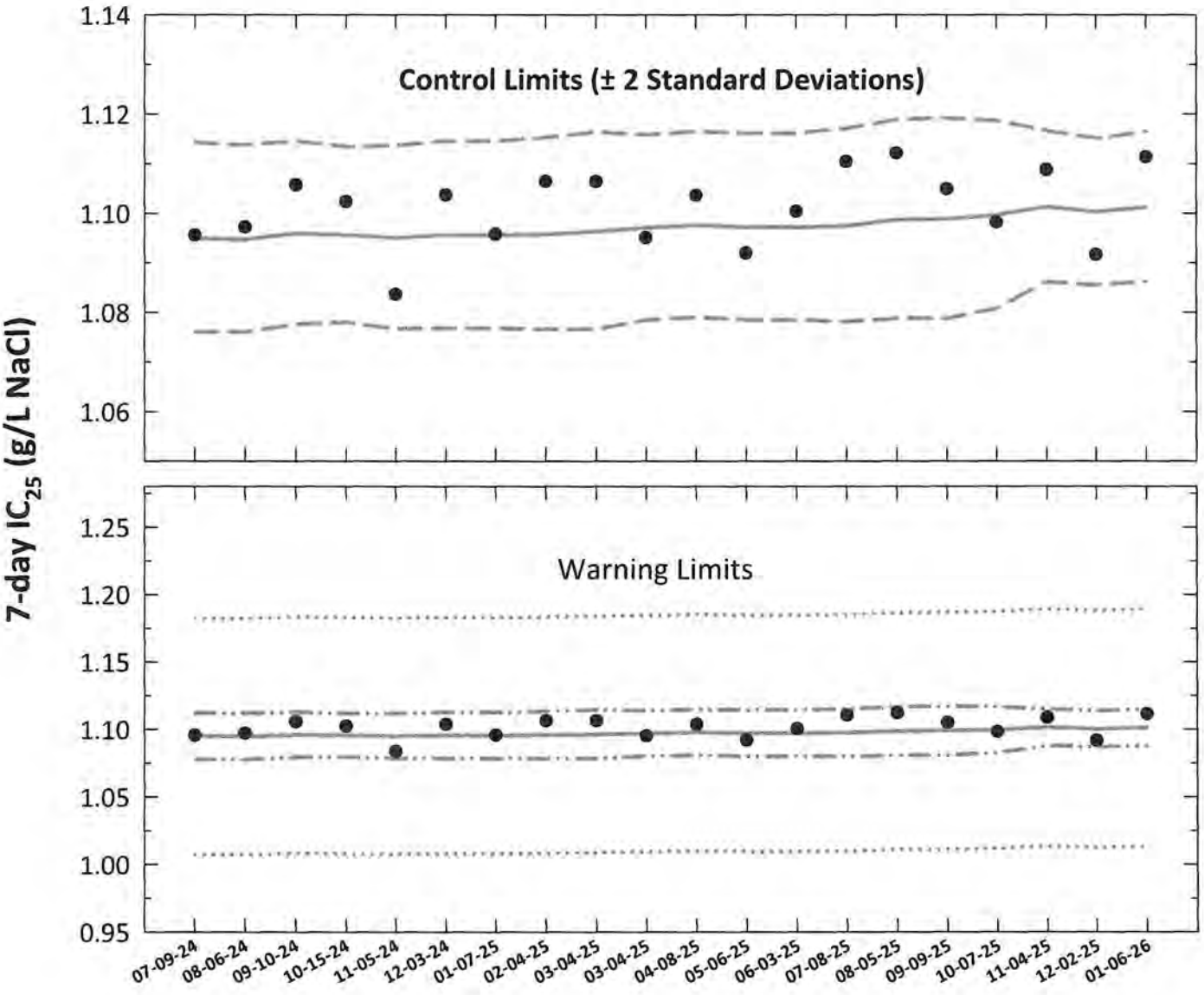


Dose-Response Plot



Environmental Testing Solutions, Inc.

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	07-09-24	1.0956	0.0396	0.0394	0.0038	1.0949	1.0760	1.1142	1.0777	1.1122	1.0073	1.1825
2	08-06-24	1.0972	0.0403	0.0393	0.0037	1.0947	1.0760	1.1138	1.0776	1.1119	1.0072	1.1823
3	09-10-24	1.1057	0.0436	0.0398	0.0037	1.0960	1.0777	1.1146	1.0793	1.1126	1.0083	1.1836
4	10-15-24	1.1024	0.0423	0.0397	0.0035	1.0956	1.0781	1.1135	1.0796	1.1116	1.0080	1.1833
5	11-05-24	1.0837	0.0349	0.0394	0.0037	1.0951	1.0767	1.1137	1.0783	1.1118	1.0075	1.1827
6	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
7	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
8	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
9	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841
10	03-04-25	1.0951	0.0394	0.0402	0.0037	1.0971	1.0786	1.1159	1.0802	1.1139	1.0093	1.1848
11	04-08-25	1.1036	0.0428	0.0405	0.0037	1.0976	1.0791	1.1165	1.0807	1.1145	1.0098	1.1854
12	05-06-25	1.0920	0.0382	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
13	06-03-25	1.1004	0.0416	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
14	07-08-25	1.1104	0.0455	0.0404	0.0038	1.0975	1.0782	1.1171	1.0799	1.1151	1.0097	1.1853
15	08-05-25	1.1121	0.0462	0.0409	0.0040	1.0987	1.0788	1.1189	1.0806	1.1167	1.0108	1.1866
16	09-09-25	1.1049	0.0433	0.0410	0.0040	1.0989	1.0789	1.1193	1.0807	1.1171	1.0110	1.1868
17	10-07-25	1.0982	0.0407	0.0412	0.0037	1.0996	1.0808	1.1187	1.0825	1.1167	1.0116	1.1876
18	11-04-25	1.1088	0.0448	0.0419	0.0030	1.1013	1.0862	1.1166	1.0876	1.1150	1.0132	1.1894
19	12-02-25	1.0917	0.0381	0.0415	0.0029	1.1003	1.0856	1.1152	1.0869	1.1136	1.0123	1.1883
20	01-06-26	1.1113	0.0458	0.0419	0.0030	1.1012	1.0863	1.1164	1.0877	1.1148	1.0131	1.1893

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.

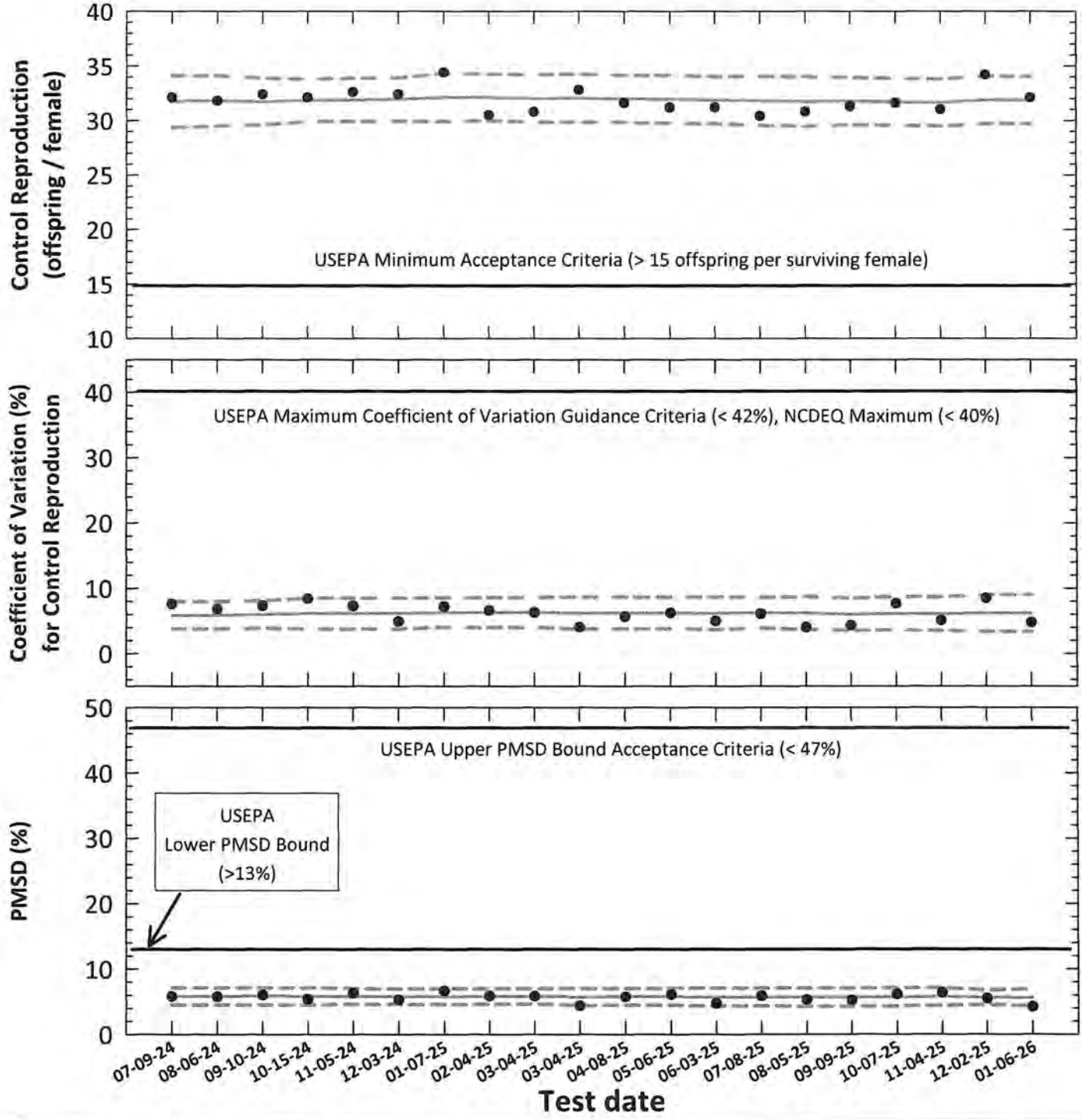
Entered and Reviewed by Jim Sumner

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	Control Reproduction		Test										
		Survival	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	07-09-24	100	32.1	7.6	1.846	5.8	31.7	29.4	34.1	5.9	3.8	7.9	5.8	4.5	7.1
2	08-06-24	100	31.8	6.8	1.827	5.7	31.8	29.5	34.1	5.9	3.8	7.9	5.8	4.5	7.1
3	09-10-24	100	32.4	7.3	1.947	6.0	31.7	29.6	33.9	6.0	3.9	8.1	5.8	4.5	7.2
4	10-15-24	100	32.1	8.4	1.715	5.3	31.9	29.9	33.8	6.1	3.8	8.5	5.8	4.5	7.2
5	11-05-24	100	32.6	7.3	2.050	6.3	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6	7.0
6	12-03-24	100	32.4	4.9	1.708	5.3	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
7	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
8	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
9	03-04-25	100	30.8	6.3	1.797	5.8	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0
10	03-04-25	100	32.8	4.0	1.430	4.4	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4	7.0
11	04-08-25	100	31.6	5.6	1.806	5.7	32.0	29.8	34.1	6.2	3.8	8.7	5.7	4.4	7.0
12	05-06-25	100	31.2	6.2	1.889	6.1	32.0	29.8	34.1	6.2	3.8	8.6	5.7	4.4	7.0
13	06-03-25	100	31.2	5.0	1.470	4.7	31.9	29.7	34.0	6.1	3.6	8.6	5.7	4.3	7.1
14	07-08-25	100	30.4	6.0	1.776	5.8	31.8	29.6	34.1	6.2	3.9	8.6	5.7	4.3	7.1
15	08-05-25	100	30.8	4.0	1.623	5.3	31.7	29.5	34.0	6.2	3.6	8.7	5.7	4.3	7.1
16	09-09-25	100	31.3	4.3	1.622	5.2	31.8	29.6	34.0	6.0	3.5	8.5	5.7	4.3	7.0
17	10-07-25	100	31.6	7.6	1.925	6.1	31.7	29.6	33.9	6.1	3.6	8.7	5.7	4.3	7.1
18	11-04-25	100	31.0	5.0	1.966	6.3	31.7	29.5	33.8	6.1	3.5	8.6	5.7	4.4	7.1
19	12-02-25	100	34.2	8.5	1.866	5.5	31.9	29.7	34.1	6.2	3.3	9.0	5.6	4.5	6.8
20	01-06-26	100	32.1	4.7	1.354	4.2	31.9	29.7	34.0	6.2	3.3	9.0	5.6	4.3	6.9

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

ETS
Environmental Testing Solutions, Inc.

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

CdNaClCR #: 319

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2433				
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:	01-06-26 0630 TO 0810									
Culture board:	12-30-25 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	2	10	11	12	18	23	24	28	29
Transfer vessel information:	pH (S.U.): 8.10 Temperature (°C): 25.0									
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	01-06-26	0822	12-24-25	12-24-25	12-30-25 A	JH
1	01-07-26	0625	↓	↓	↓	JH
2	01-08-26	0630	↓	↓	↓	JH
3	01-09-26	0630	↓	↓	12-30-25 B	JH
4	01-10-26	0830	↓	↓	↓	JH
5	01-11-26	0640	↓	↓	01-06-25 A ~~12-30-25 B~~ 01-11-26	JH
6	01-12-26	0742	↓	↓	↓	JH
7	01-13-26	0630				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-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	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:	32.1	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	4.7%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1111.3

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 319

CONTROL ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	5	5	5	5	4	3	5	5	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	10	13	12	12	10	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	17	15	17	13	18	15	19	19	17	16
Total young produced		33	30	32	31	35	31	32	34	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:	32.1

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	5	3	5	5	5	6	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	11	13	10	13	12	12	11	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	15	16	19	17	16	13	18	15	17
Total young produced		33	30	34	32	35	33	30	35	31	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.6
% Reduction from Control:	-1.67%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 319**

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	6	5	5	3	5	5	4	4	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	13	12	10	12	10	12	12	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	14	13	18	16	15	19	16	16	15
Total young produced		32	32	31	33	31	32	33	32	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:	32.0
% Reduction from Control:	0.31

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	5	4	4	4	4	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	9	11	11	10	12	10	10	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	19	18	17	18	15	16	18	14	17
Total young produced		32	33	33	33	32	31	30	32	31	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.0
% Reduction from Control:	0.31

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 319**

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	3	5	5	5	3	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	5	9	6	10	7	8	5	11	7	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	10	5	6	6	5	7	8	3	7	9
Total young produced		19	17	17	21	17	18	17	18	18	19
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	18.1
% Reduction from Control:	43.6%

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	3	1	2	4	2	2	2	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	0	0	0	2	0	0	0	0	0	0
Total young produced		2	1	3	3	2	4	2	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:	2.4
% Reduction from Control:	92.5%

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	5	5	5	5	4	3	5	5	3	44
5	12	10	10	13	12	12	10	10	10	12	111
6	0	0	0	0	0	0	0	0	0	0	0
7	17	15	17	13	18	15	19	19	17	16	166
Total	**33**	**30**	**32**	**31**	**35**	**31**	**32**	**34**	**32**	**31**	**321**

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	5	4	4	4	4	6	4	45
5	12	9	11	11	10	12	10	10	11	12	108
6	0	0	0	0	0	0	0	0	0	0	0
7	15	19	18	17	18	15	16	18	14	17	167
Total	**32**	**33**	**33**	**33**	**32**	**31**	**30**	**32**	**31**	**33**	**320**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	5	3	5	5	5	6	4	6	48
5	10	11	13	10	13	12	12	11	12	10	114
6	0	0	0	0	0	0	0	0	0	0	0
7	18	15	16	19	17	16	13	18	15	17	164
Total	**33**	**30**	**34**	**32**	**35**	**33**	**30**	**35**	**31**	**33**	**326**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	5	5	5	3	4	4	4	4	41
5	5	9	6	10	7	8	5	11	7	6	74
6	0	0	0	0	0	0	0	0	0	0	0
7	10	5	6	6	5	7	8	3	7	9	66
Total	**19**	**17**	**17**	**21**	**17**	**18**	**17**	**18**	**18**	**19**	**181**

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	6	5	5	3	5	5	4	4	6	4	47
5	12	13	13	12	10	12	10	12	12	11	117
6	0	0	0	0	0	0	0	0	0	0	0
7	14	14	13	18	16	15	19	16	16	15	156
Total	**32**	**32**	**31**	**33**	**31**	**32**	**33**	**32**	**34**	**30**	**320**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	3	1	2	4	2	2	2	3	22
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	2	0	0	0	0	0	0	2
Total	**2**	**1**	**3**	**3**	**2**	**4**	**2**	**2**	**2**	**3**	**24**

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: 319

Test dates: January 06-13, 2026

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	33	30	32	31	35	31	32	34	32	31	100	32.1	4.7	Not applicable
600	33	30	34	32	35	33	30	35	31	33	100	32.6	5.6	-1.6
800	32	32	31	33	31	32	33	32	34	30	100	32.0	3.6	0.3
1000	32	33	33	33	32	31	30	32	31	33	100	32.0	3.3	0.3
1200	19	17	17	21	17	18	17	18	18	19	100	18.1	7.1	43.6
1400	2	1	3	3	2	4	2	2	2	3	100	2.4	35.1	92.5

Dunnett's MSD value: 1.354

PMSD: 4.2

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

Upper PMSD bound determined by USEPA (90^{th} 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

Entered and Reviewed by Jim Sawyer

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	1/6/2026	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	1/13/2026	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	33.000	30.000	32.000	31.000	35.000	31.000	32.000	34.000	32.000	31.000
600	33.000	30.000	34.000	32.000	35.000	33.000	30.000	35.000	31.000	33.000
800	32.000	32.000	31.000	33.000	31.000	32.000	33.000	32.000	34.000	30.000
1000	32.000	33.000	33.000	33.000	32.000	31.000	30.000	32.000	31.000	33.000
1200	19.000	17.000	17.000	21.000	17.000	18.000	17.000	18.000	18.000	19.000
1400	2.000	1.000	3.000	3.000	2.000	4.000	2.000	2.000	2.000	3.000

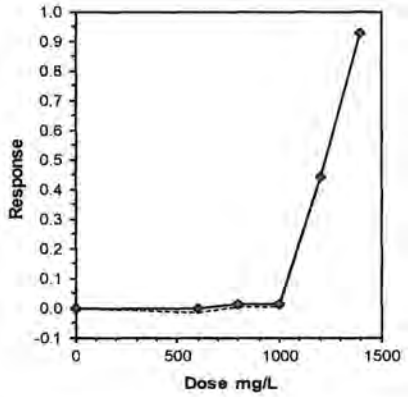
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	32.100	1.0000	32.100	30.000	35.000	4.747	10				32.350	1.0000
600	32.600	1.0156	32.600	30.000	35.000	5.638	10	-0.845	2.287	1.354	32.350	1.0000
800	32.000	0.9969	32.000	30.000	34.000	3.608	10	0.169	2.287	1.354	32.000	0.9892
1000	32.000	0.9969	32.000	30.000	33.000	3.294	10	0.169	2.287	1.354	32.000	0.9892
*1200	18.100	0.5639	18.100	17.000	21.000	7.109	10	23.652	2.287	1.354	18.100	0.5595
*1400	2.400	0.0748	2.400	1.000	4.000	35.136	10	50.176	2.287	1.354	2.400	0.0742

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.91533	1.035	0.22488	-0.0684
Bartlett's Test indicates equal variances (p = 0.26)	6.45355	15.0863		

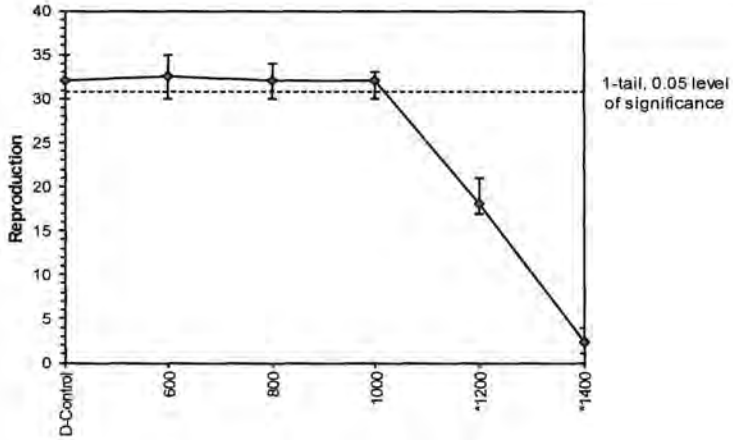
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.35353	0.04217	1528.87	1.75185	0.0E+00	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1018.24	7.14884	1003.56	1023.58	-3.8382
IC10	1041.51	5.29082	1027.09	1047.31	-0.6393
IC15	1064.78	5.00737	1051.53	1071.27	-0.5471
IC20	1088.06	4.85845	1076.21	1095.03	-0.3926
IC25	1111.33	4.85648	1099.99	1119.11	-0.1983
IC40	1181.15	5.67693	1171.1	1192.67	0.2520
IC50	1224.52	4.64123	1215.79	1233.23	0.1066



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 319**

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	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.84	7.91	7.73	7.84	7.79	7.84
	Dissolved oxygen (mg/L)	8.5	8.0	8.2	8.2	8.4	8.0
	Conductivity (µmhos/cm)	289		305		300	
	Alkalinity (mg $CaCO_3$/L)	63					
	Hardness (mg $CaCO_3$/L)	90				01-13 26 M	
	Temperature (°C)	25.2	25.3	25.0	25.1	24.8	25.1
600 mg NaCl/L	pH (S.U.)	7.91	7.92	7.88	7.87	7.87	7.85
	Dissolved oxygen (mg/L)	8.5	8.0	8.2	8.2	8.4	8.0
	Conductivity (µmhos/cm)	1320		1400		1400	
	Temperature (°C)	25.0	25.0	25.2	24.9	24.7	24.8
800 mg NaCl/L	pH (S.U.)	7.91	7.92	7.88	7.87	7.87	7.84
	Dissolved oxygen (mg/L)	8.5	8.0	8.2	8.2	8.4	8.0
	Conductivity (µmhos/cm)	1460		1720		1770	
	Temperature (°C)	25.0	25.2	25.2	25.2	24.9	24.8
1000 mg NaCl/L	pH (S.U.)	7.92	7.93	7.89	7.87	7.90	7.85
	Dissolved oxygen (mg/L)	8.5	8.1	8.2	8.1	8.3	8.0
	Conductivity (µmhos/cm)	2010		2120		2140	
	Temperature (°C)	25.0	25.2	25.2	25.2	24.9	24.9
1200 mg NaCl/L	pH (S.U.)	7.95	7.94	7.90	7.88	7.91	7.87
	Dissolved oxygen (mg/L)	8.5	8.1	8.2	8.1	8.3	8.0
	Conductivity (µmhos/cm)	2350		2500		2500	
	Temperature (°C)	25.2	25.4	25.0	25.0	24.8	25.0
1400 mg NaCl/L	pH (S.U.)	7.96	7.94	7.91	7.88	7.91	7.86
	Dissolved oxygen (mg/L)	8.4	8.0	8.2	8.2	8.3	7.9
	Conductivity (µmhos/cm)	2660		2830		2810	
	Temperature (°C)	25.2	25.1	25.1	25.3	24.9	24.8
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

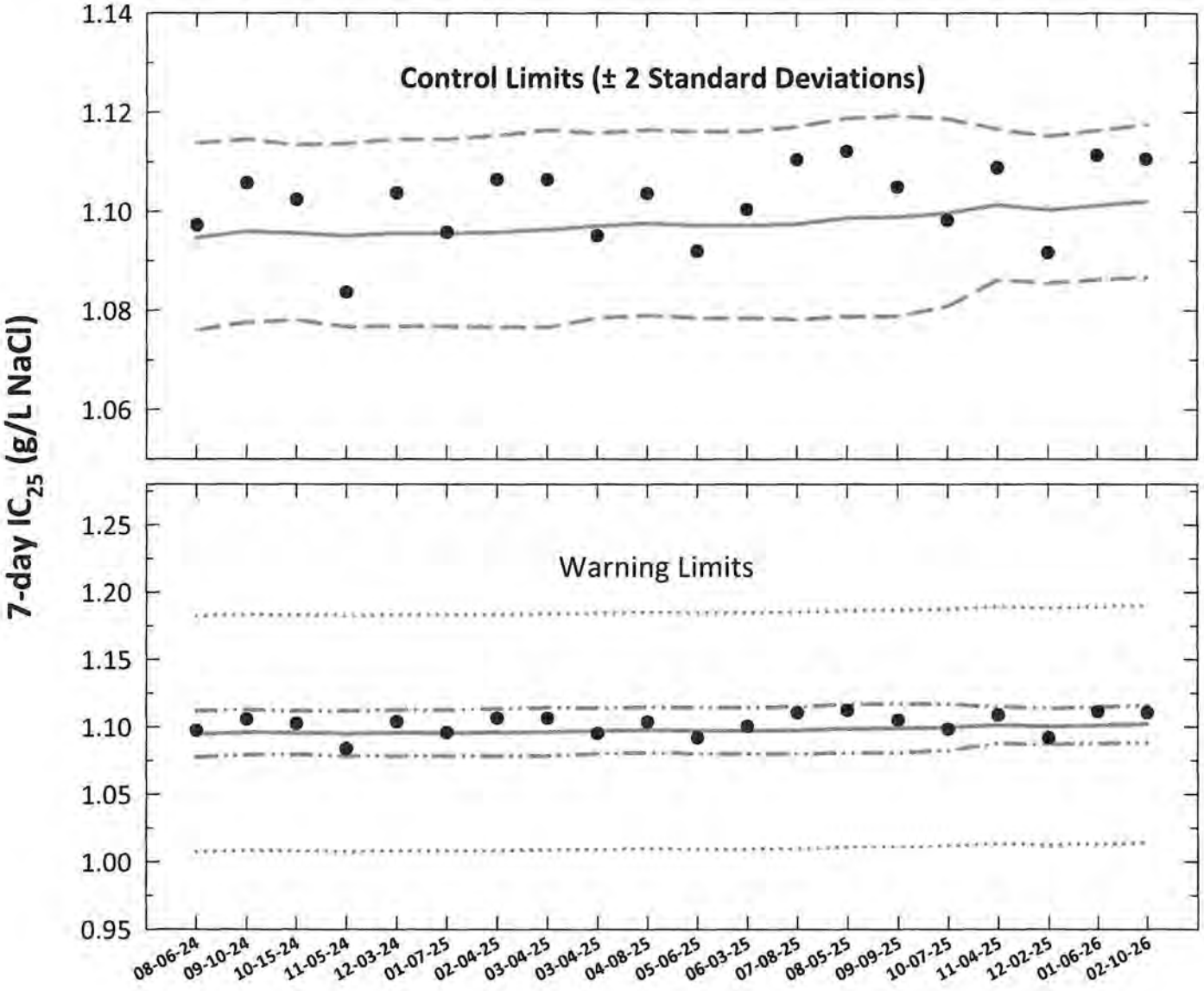
Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 319

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	K
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.75	7.91	7.47	7.98 (8.0)	7.84	7.97	7.89	7.87
	Dissolved oxygen (mg/L)	8.3	8.2	8.5	*~~7.2~~ (8.0)	8.3	8.2	8.4	7.8
	Conductivity (µmhos/cm)	298		280		298		301	
	Alkalinity (mg $CaCO_3$/L)	63				62			
	Hardness (mg $CaCO_3$/L)	90			*01-13-26 K	86			*01-13-26 K
	Temperature (°C)	24.8	25.1	24.9	25.0	24.8	25.1	24.7	25.1
600 mg NaCl/L	pH (S.U.)	7.84	7.94	7.79	8.01	7.91	7.99	8.00	7.89
	Dissolved oxygen (mg/L)	8.3	8.2	8.5	*~~6.8~~ (8.0)	8.2	8.2	8.5	7.7
	Conductivity (µmhos/cm)	1370		1330		1370		1340	
	Temperature (°C)	24.9	25.3	25.0	25.2	24.9	24.9	24.8	24.9
800 mg NaCl/L	pH (S.U.)	7.85	7.94	7.89	8.03	8.03	8.00	8.01	7.90
	Dissolved oxygen (mg/L)	8.3	8.1	8.4	*~~6.9~~ (8.0)	8.2	8.2	8.5	7.6
	Conductivity (µmhos/cm)	1740		1680		1730		1740	
	Temperature (°C)	25.0	25.2	25.0	25.2	24.9	24.9	24.9	24.9
1000 mg NaCl/L	pH (S.U.)	7.85	7.95	7.91	8.03	8.04	8.01	8.02	7.90
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	*~~7.0~~ (8.0)	8.2	8.2	8.4	7.6
	Conductivity (µmhos/cm)	2100		2030		2100		2120	
	Temperature (°C)	24.9	25.2	24.9	24.9	25.0	25.2	24.9	24.9
1200 mg NaCl/L	pH (S.U.)	7.85	7.95	7.92	8.04	8.04	8.01	8.03	7.92
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	*~~6.9~~ (7.9)	8.2	8.2	8.4	7.6
	Conductivity (µmhos/cm)	2470		2360		2460		2480	
	Temperature (°C)	24.9	25.0	24.9	24.9	24.9	25.2	24.9	25.2
1400 mg NaCl/L	pH (S.U.)	7.86	7.96	7.93	8.03	8.05	8.01	8.04	7.93
	Dissolved oxygen (mg/L)	8.2	8.1	8.3	7.9	8.2	8.1	8.4	7.6
	Conductivity (µmhos/cm)	2800		2700		2790		2810	
	Temperature (°C)	24.9	25.0	25.1	24.9	24.9	25.2	24.9	25.0
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

* BSL 01.11.26 verified correct

ETS
Environmental Testing Solutions, Inc.

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	08-06-24	1.0972	0.0403	0.0393	0.0037	1.0947	1.0760	1.1138	1.0776	1.1119	1.0072	1.1823
2	09-10-24	1.1057	0.0436	0.0398	0.0037	1.0960	1.0777	1.1146	1.0793	1.1126	1.0083	1.1836
3	10-15-24	1.1024	0.0423	0.0397	0.0035	1.0956	1.0781	1.1135	1.0796	1.1116	1.0080	1.1833
4	11-05-24	1.0837	0.0349	0.0394	0.0037	1.0951	1.0767	1.1137	1.0783	1.1118	1.0075	1.1827
5	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
6	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
7	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
8	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841
9	03-04-25	1.0951	0.0394	0.0402	0.0037	1.0971	1.0786	1.1159	1.0802	1.1139	1.0093	1.1848
10	04-08-25	1.1036	0.0428	0.0405	0.0037	1.0976	1.0791	1.1165	1.0807	1.1145	1.0098	1.1854
11	05-06-25	1.0920	0.0382	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
12	06-03-25	1.1004	0.0416	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
13	07-08-25	1.1104	0.0455	0.0404	0.0038	1.0975	1.0782	1.1171	1.0799	1.1151	1.0097	1.1853
14	08-05-25	1.1121	0.0462	0.0409	0.0040	1.0987	1.0788	1.1189	1.0806	1.1167	1.0108	1.1866
15	09-09-25	1.1049	0.0433	0.0410	0.0040	1.0989	1.0789	1.1193	1.0807	1.1171	1.0110	1.1868
16	10-07-25	1.0982	0.0407	0.0412	0.0037	1.0996	1.0808	1.1187	1.0825	1.1167	1.0116	1.1876
17	11-04-25	1.1088	0.0448	0.0419	0.0030	1.1013	1.0862	1.1166	1.0876	1.1150	1.0132	1.1894
18	12-02-25	1.0917	0.0381	0.0415	0.0029	1.1003	1.0856	1.1152	1.0869	1.1136	1.0123	1.1883
19	01-06-26	1.1113	0.0458	0.0419	0.0030	1.1012	1.0863	1.1164	1.0877	1.1148	1.0131	1.1893
20	02-10-26	1.1106	0.0455	0.0422	0.0030	1.1020	1.0867	1.1175	1.0881	1.1158	1.0138	1.1901

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.

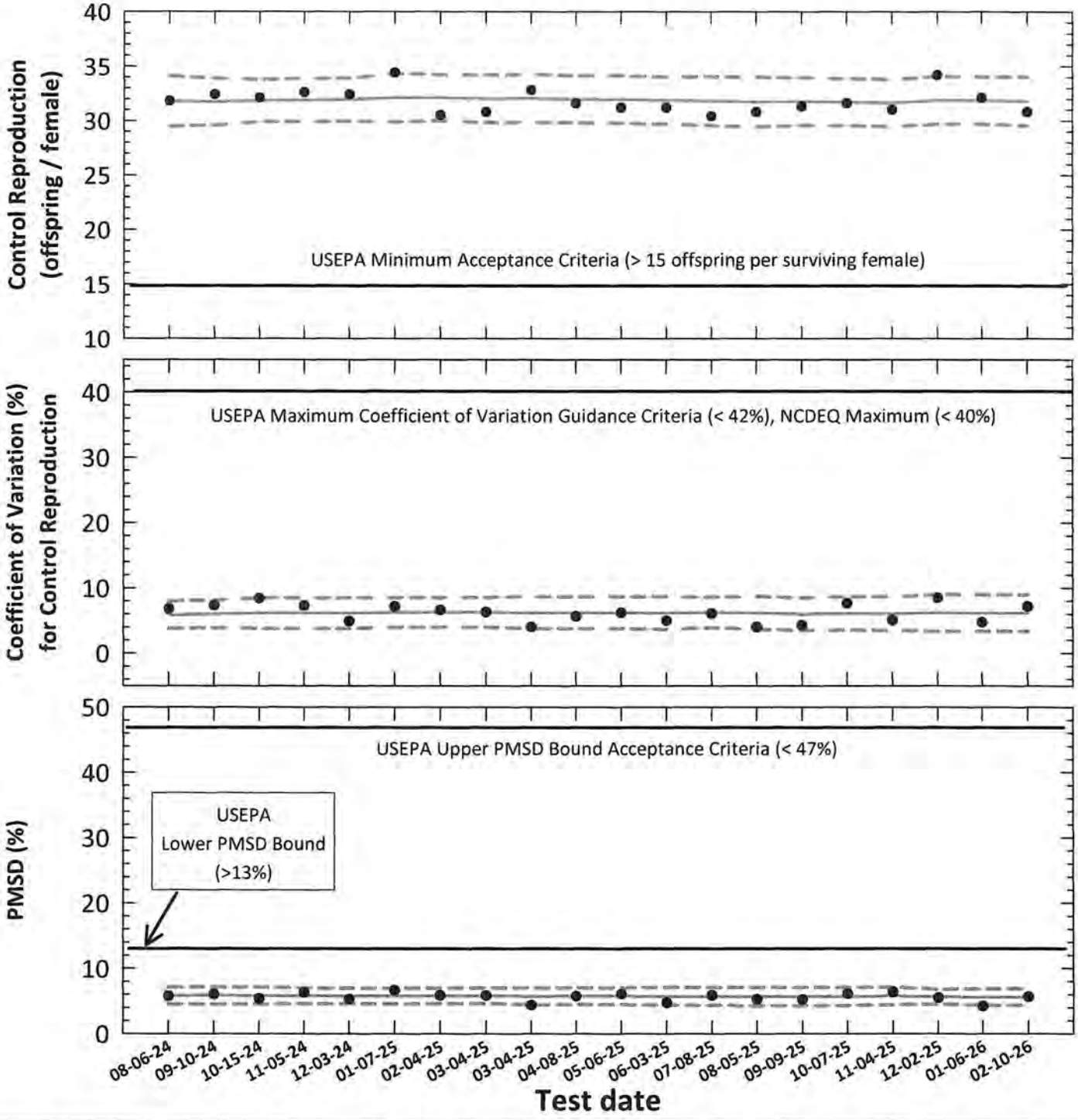
Entered and Reviewed by Jim Sumner

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	Control Reproduction		Test										
		Survival	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	08-06-24	100	31.8	6.8	1.827	5.7	31.8	29.5	34.1	5.9	3.8	7.9	5.8	4.5	7.1
2	09-10-24	100	32.4	7.3	1.947	6.0	31.7	29.6	33.9	6.0	3.9	8.1	5.8	4.5	7.2
3	10-15-24	100	32.1	8.4	1.715	5.3	31.9	29.9	33.8	6.1	3.8	8.5	5.8	4.5	7.2
4	11-05-24	100	32.6	7.3	2.050	6.3	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6	7.0
5	12-03-24	100	32.4	4.9	1.708	5.3	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
6	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
7	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
8	03-04-25	100	30.8	6.3	1.797	5.8	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0
9	03-04-25	100	32.8	4.0	1.430	4.4	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4	7.0
10	04-08-25	100	31.6	5.6	1.806	5.7	32.0	29.8	34.1	6.2	3.8	8.7	5.7	4.4	7.0
11	05-06-25	100	31.2	6.2	1.889	6.1	32.0	29.8	34.1	6.2	3.8	8.6	5.7	4.4	7.0
12	06-03-25	100	31.2	5.0	1.470	4.7	31.9	29.7	34.0	6.1	3.6	8.6	5.7	4.3	7.1
13	07-08-25	100	30.4	6.0	1.776	5.8	31.8	29.6	34.1	6.2	3.9	8.6	5.7	4.3	7.1
14	08-05-25	100	30.8	4.0	1.623	5.3	31.7	29.5	34.0	6.2	3.6	8.7	5.7	4.3	7.1
15	09-09-25	100	31.3	4.3	1.622	5.2	31.8	29.6	34.0	6.0	3.5	8.5	5.7	4.3	7.0
16	10-07-25	100	31.6	7.6	1.925	6.1	31.7	29.6	33.9	6.1	3.6	8.7	5.7	4.3	7.1
17	11-04-25	100	31.0	5.0	1.966	6.3	31.7	29.5	33.8	6.1	3.5	8.6	5.7	4.4	7.1
18	12-02-25	100	34.2	8.5	1.866	5.5	31.9	29.7	34.1	6.2	3.3	9.0	5.6	4.5	6.8
19	01-06-26	100	32.1	4.7	1.354	4.2	31.9	29.7	34.0	6.2	3.3	9.0	5.6	4.3	6.9
20	02-10-26	100	30.8	7.1	1.734	5.6	31.8	29.6	34.0	6.1	3.3	8.9	5.6	4.3	6.9

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 Summer

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 320

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2451				
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:	02·10·26 0540 TO 0800									
Culture board:	02·03·26 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	2	6	7	11	13	17	18	19	22
Transfer vessel information:	pH (S.U.): 7.90 Temperature (°C): 25.1									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	BLACK
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	02-10-26	0810	01-28-26	12·24·25	02·04·26 B	
1	02-11-26	0610	↓	↓	↓	
2	02-12-26	0615			02·09·26 A	
3	02-13-26	0610			↓	
4	02-14-26	0700			02·09·26 B	
5	02-15-26	0700			↓	
6	02-16-26	0625	↓	↓		
7	02-17-26	0700				

*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-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	13066485

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% of Male Adults:	0%	≤ 20%	**7-day LC_{50}** (mg/L NaCl)	71400
% 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:	7.17%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1110.6

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 320

CONTROL ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	4	3	3	4	5	5	5	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	13	12	12	12	10	10	13	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	15	16	15	17	12	15	15	15
Total young produced		30	34	31	31	30	31	27	33	33	28
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	4	4	4	5	4	5	3	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	10	12	10	10	11	12	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	15	16	17	17	14	17	18	15	16
Total young produced		34	32	30	34	31	29	31	35	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:	32.0
% Reduction from Control:	-3.9%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 320

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 ~~0~~ 5 CO	0	4	4	4	5	6	6	4	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	12	12	13	10	10	10	12	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	17	14	17	13	16	16	16	15	13
Total young produced		33	33	30	33	30	31	32	32	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:	31.7
% Reduction from Control:	-2.9%

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	2	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	5	4	3	4	4	4	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	11	10	12	10	12	12	11	12	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	14	14	17	15	15	17	16	14	16
Total young produced		30	29	29	33	28	31	33	31	32	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.7
% Reduction from Control:	0.3%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 320**

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.9
% Reduction from Control:	41.9%

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2-5
% Reduction from Control:	91.9%

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	5	4	4	3	3	4	5	5	5	3	41
5	10	13	12	12	12	10	10	13	13	10	115
6	0	0	0	0	0	0	0	0	0	0	0
7	15	17	15	16	15	17	12	15	15	15	152
Total	**30**	**34**	**31**	**31**	**30**	**31**	**27**	**33**	**33**	**28**	**308**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	5	4	3	4	4	4	6	4	42
5	11	11	10	12	10	12	12	11	12	11	112
6	0	0	0	0	0	0	0	0	0	0	0
7	15	14	14	17	15	15	17	16	14	16	153
Total	**30**	**29**	**29**	**33**	**28**	**31**	**33**	**31**	**32**	**31**	**307**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	4	5	4	5	3	5	5	4	43
5	12	13	10	12	10	10	11	12	12	12	114
6	0	0	0	0	0	0	0	0	0	0	0
7	18	15	16	17	17	14	17	18	15	16	163
Total	**34**	**32**	**30**	**34**	**31**	**29**	**31**	**35**	**32**	**32**	**320**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	4	3	3	4	4	5	5	3	39
5	10	7	10	8	9	5	7	7	9	7	79
6	0	0	0	0	0	0	0	0	0	0	0
7	3	5	6	7	7	10	4	6	4	9	61
Total	**18**	**15**	**20**	**18**	**19**	**19**	**15**	**18**	**18**	**19**	**179**

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	4	4	5	6	6	4	6	48
5	12	11	12	12	13	10	10	10	12	13	115
6	0	0	0	0	0	0	0	0	0	0	0
7	17	17	14	17	13	16	16	16	15	13	154
Total	**33**	**33**	**30**	**33**	**30**	**31**	**32**	**32**	**31**	**32**	**317**

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	4	4	4	2	1	3	1	1	1	3	24
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	1	0	0	0	0	0	0	1
Total	**4**	**4**	**4**	**3**	**1**	**3**	**1**	**1**	**1**	**3**	**25**

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: 320

Test dates: February 10-17, 2026

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	34	31	31	30	31	27	33	33	28	100	30.8	7.1	Not applicable
600	34	32	30	34	31	29	31	35	32	32	100	32.0	5.9	-3.9
800	33	33	30	33	30	31	32	32	31	32	100	31.7	3.7	-2.9
1000	30	29	29	33	28	31	33	31	32	31	100	30.7	5.5	0.3
1200	18	15	20	18	19	19	15	18	18	19	100	17.9	9.3	41.9
1400	4	4	4	3	1	3	1	1	1	3	100	2.5	54.2	91.9

Dunnett's MSD value: 1.734

PMSD: 5.6

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

Upper PMSD bound determined by USEPA (90^{th} 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

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	2/10/2026	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	2/17/2026	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	34.000	31.000	31.000	30.000	31.000	27.000	33.000	33.000	28.000
600	34.000	32.000	30.000	34.000	31.000	29.000	31.000	35.000	32.000	32.000
800	33.000	33.000	30.000	33.000	30.000	31.000	32.000	32.000	31.000	32.000
1000	30.000	29.000	29.000	33.000	28.000	31.000	33.000	31.000	32.000	31.000
1200	18.000	15.000	20.000	18.000	19.000	19.000	15.000	18.000	18.000	19.000
1400	4.000	4.000	4.000	3.000	1.000	3.000	1.000	1.000	1.000	3.000

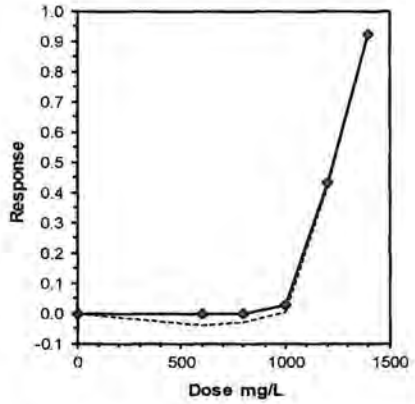
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	30.800	1.0000	30.800	27.000	34.000	7.146	10			31.500	1.0000
600	32.000	1.0390	32.000	29.000	35.000	5.893	10	121.00	75.00	31.500	1.0000
800	31.700	1.0292	31.700	30.000	33.000	3.658	10	117.00	75.00	31.500	1.0000
1000	30.700	0.9968	30.700	28.000	33.000	5.547	10	103.00	75.00	30.700	0.9746
*1200	17.900	0.5812	17.900	15.000	20.000	9.292	10	55.00	75.00	17.900	0.5683
*1400	2.500	0.0812	2.500	1.000	4.000	54.160	10	55.00	75.00	2.500	0.0794

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.04609	1.035	-0.2604	-0.4738
Bartlett's Test indicates equal variances (p = 0.50)	4.3444	15.0863		

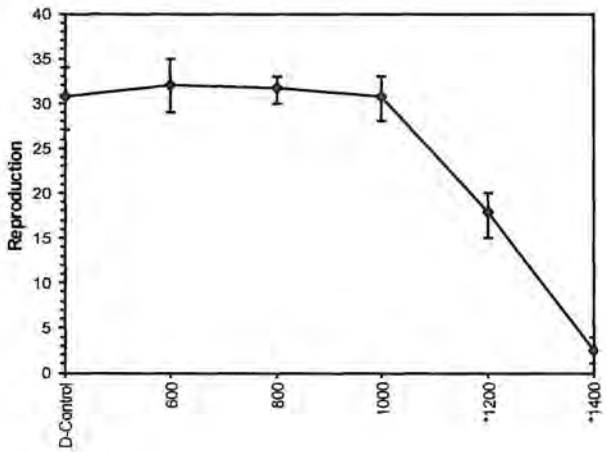
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1000	1200	1095.45	
Treatments vs D-Control				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1012.11	20.9497	939.698	1022.78	-3.1602
IC10	1036.72	7.50783	1018.76	1046.51	-0.3700
IC15	1061.33	6.89539	1045.16	1070.82	-0.3544
IC20	1085.94	6.49365	1070.79	1094.7	-0.3416
IC25	1110.55	6.34277	1096.31	1119.57	-0.3309
IC40	1184.38	7.42013	1168.36	1197.1	-0.2374
IC50	1227.92	5.99586	1214.03	1237.39	-0.5601



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Used for PMSD calculation only.

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	2/10/2026	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	2/17/2026	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:					

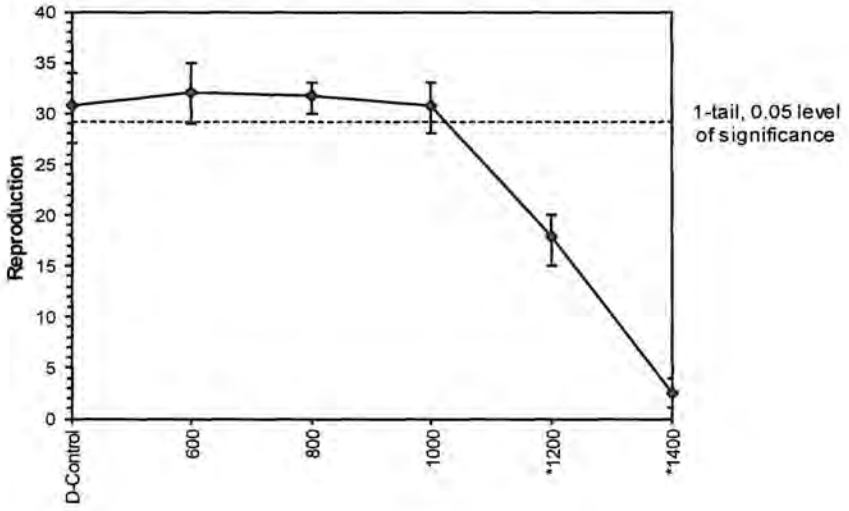
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	30.000	34.000	31.000	31.000	30.000	31.000	27.000	33.000	33.000	28.000
600	34.000	32.000	30.000	34.000	31.000	29.000	31.000	35.000	32.000	32.000
800	33.000	33.000	30.000	33.000	30.000	31.000	32.000	32.000	31.000	32.000
1000	30.000	29.000	29.000	33.000	28.000	31.000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

			Transform: Untransformed						1-Tailed	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	30.800	1.0000	30.800	27.000	34.000	7.146	10			
600	32.000	1.0390	32.000	29.000	35.000	5.893	10	-1.583	2.287	1.734
800	31.700	1.0292	31.700	30.000	33.000	3.658	10	-1.187	2.287	1.734
1000	30.700	0.9968	30.700	28.000	33.000	5.547	10	0.132	2.287	1.734
*1200	17.900	0.5812	17.900	15.000	20.000	9.292	10	17.015	2.287	1.734
*1400	2.500	0.0812	2.500	1.000	4.000	54.160	10	37.327	2.287	1.734

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.04609	1.035	-0.2604	-0.4738
Bartlett's Test indicates equal variances (p = 0.50)	4.3444	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	1200	1095.45		1.73367	0.05629	1426.91	2.87407	8.0E-44	5, 54

Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 320

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	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.82	7.86	7.78	7.88	7.83	7.92
	Dissolved oxygen (mg/L)	8.4	8.0	8.4	8.2	8.5	8.1
	Conductivity (µmhos/cm)	295		302		295	
	Alkalinity (mg $CaCO_3$/L)	63				60	
	Hardness (mg $CaCO_3$/L)	86				84	
	Temperature (°C)	24.9	25.2	24.8	25.1	24.8	24.9
600 mg NaCl/L	pH (S.U.)	7.92	7.87	7.84	7.89	7.89	7.93
	Dissolved oxygen (mg/L)	8.5	8.0	8.3	8.1	8.5	8.1
	Conductivity (µmhos/cm)	1380		1380		1370	
	Temperature (°C)	24.9	24.9	24.8	25.0	24.9	25.1
800 mg NaCl/L	pH (S.U.)	7.93	7.87	7.85	7.90	7.89	7.94
	Dissolved oxygen (mg/L)	8.5	8.0	8.2	8.1	8.5	8.1
	Conductivity (µmhos/cm)	1720		1750		1740	
	Temperature (°C)	25.0	24.9	24.9	25.3	24.8	24.9
1000 mg NaCl/L	pH (S.U.)	7.94	7.88	7.85	7.90	7.91	7.94
	Dissolved oxygen (mg/L)	8.6	8.1	8.2	8.1	8.5	8.1
	Conductivity (µmhos/cm)	2060		2080		2050	
	Temperature (°C)	25.1	25.1	24.7	25.3	24.8	24.9
1200 mg NaCl/L	pH (S.U.)	7.95	7.87	7.88	7.92	7.92	7.93
	Dissolved oxygen (mg/L)	8.5	8.0	8.2	8.2	8.5	8.1
	Conductivity (µmhos/cm)	2400		2420		2440	
	Temperature (°C)	25.1	25.1	24.9	25.0	24.8	25.1
1400 mg NaCl/L	pH (S.U.)	7.95	7.87	7.88	7.93	7.92	7.94
	Dissolved oxygen (mg/L)	8.4	8.0	8.2	8.2	8.5	8.1
	Conductivity (µmhos/cm)	2760		2770		2770	
	Temperature (°C)	25.0	25.1	24.9	25.0	24.8	24.8
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

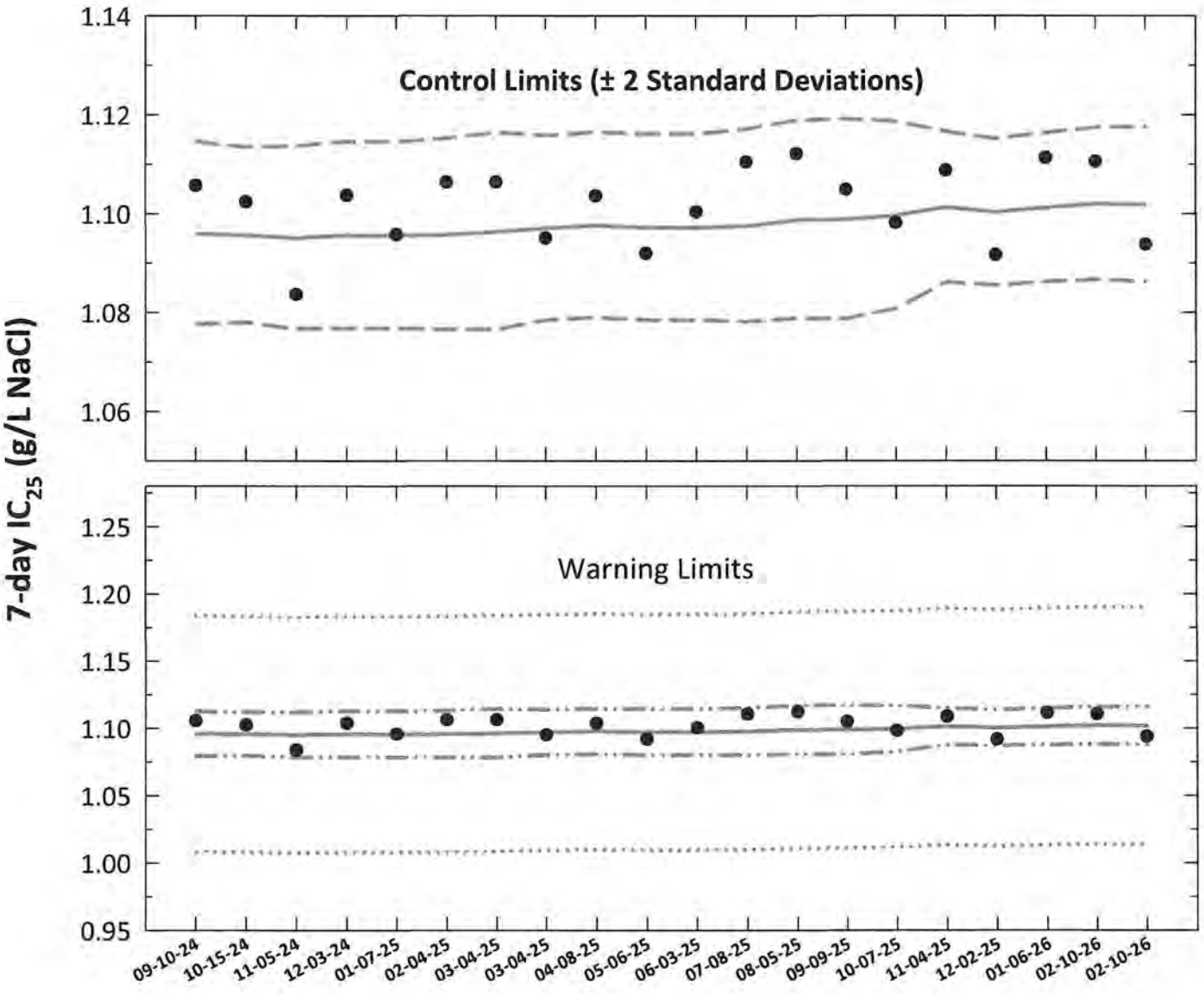
Species: ***Ceriodaphnia dubia***

CdNaClCR #: 320

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.85	7.98	7.79	8.00	7.81	7.98	7.87	8.13
	Dissolved oxygen (mg/L)	8.5	8.2	8.6	8.2	8.6	8.2	8.4	7.6
	Conductivity (µmhos/cm)	297		285		277		306	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)			88					
	Temperature (°C)	24.8	25.3	24.9	25.1	24.7	25.2	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.89	8.00	7.81	8.00	7.86	7.98	7.91	8.09
	Dissolved oxygen (mg/L)	8.4	8.3	8.6	8.2	8.6	8.2	8.4	7.6
	Conductivity (µmhos/cm)	1360		1360		1340		1430	
	Temperature (°C)	25.0	25.0	25.0	24.9	24.9	25.2	24.7	25.0
800 mg NaCl/L	pH (S.U.)	7.90	8.00	7.94	8.00	7.93	7.98	7.94	8.08
	Dissolved oxygen (mg/L)	8.5	8.2	8.5	8.1	8.5	8.2	8.4	7.6
	Conductivity (µmhos/cm)	1720		1690		1650		1790	
	Temperature (°C)	25.0	25.2	25.0	25.2	24.9	25.0	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	7.90	8.00	7.96	8.01	7.98	7.98	7.94	8.06
	Dissolved oxygen (mg/L)	8.4	8.2	8.6	8.1	8.5	8.2	8.4	7.6
	Conductivity (µmhos/cm)	2040		2030		1990		2110	
	Temperature (°C)	24.8	25.2	25.0	25.0	24.8	24.8	24.9	24.9
1200 mg NaCl/L	pH (S.U.)	7.91	7.99	7.97	8.01	8.00	7.99	7.96	8.05
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.0	8.5	8.2	8.3	7.6
	Conductivity (µmhos/cm)	2400		2360		2310		2440	
	Temperature (°C)	24.8	25.1	25.2	25.0	24.8	24.8	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	7.92	7.99	7.98	8.00	8.01	7.98	7.98	8.03
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.1	8.4	8.2	8.3	7.6
	Conductivity (µmhos/cm)	2730		(2670)		2640		2790	
	Temperature (°C)	24.8	25.3	25.2	25.0	24.8	25.1	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

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



Test date

- ● **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)

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	09-10-24	1.1057	0.0436	0.0398	0.0037	1.0960	1.0777	1.1146	1.0793	1.1126	1.0083	1.1836
2	10-15-24	1.1024	0.0423	0.0397	0.0035	1.0956	1.0781	1.1135	1.0796	1.1116	1.0080	1.1833
3	11-05-24	1.0837	0.0349	0.0394	0.0037	1.0951	1.0767	1.1137	1.0783	1.1118	1.0075	1.1827
4	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
5	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
6	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
7	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841
8	03-04-25	1.0951	0.0394	0.0402	0.0037	1.0971	1.0786	1.1159	1.0802	1.1139	1.0093	1.1848
9	04-08-25	1.1036	0.0428	0.0405	0.0037	1.0976	1.0791	1.1165	1.0807	1.1145	1.0098	1.1854
10	05-06-25	1.0920	0.0382	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
11	06-03-25	1.1004	0.0416	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
12	07-08-25	1.1104	0.0455	0.0404	0.0038	1.0975	1.0782	1.1171	1.0799	1.1151	1.0097	1.1853
13	08-05-25	1.1121	0.0462	0.0409	0.0040	1.0987	1.0788	1.1189	1.0806	1.1167	1.0108	1.1866
14	09-09-25	1.1049	0.0433	0.0410	0.0040	1.0989	1.0789	1.1193	1.0807	1.1171	1.0110	1.1868
15	10-07-25	1.0982	0.0407	0.0412	0.0037	1.0996	1.0808	1.1187	1.0825	1.1167	1.0116	1.1876
16	11-04-25	1.1088	0.0448	0.0419	0.0030	1.1013	1.0862	1.1166	1.0876	1.1150	1.0132	1.1894
17	12-02-25	1.0917	0.0381	0.0415	0.0029	1.1003	1.0856	1.1152	1.0869	1.1136	1.0123	1.1883
18	01-06-26	1.1113	0.0458	0.0419	0.0030	1.1012	1.0863	1.1164	1.0877	1.1148	1.0131	1.1893
19	02-10-26	1.1106	0.0455	0.0422	0.0030	1.1020	1.0867	1.1175	1.0881	1.1158	1.0138	1.1901
20	02-10-26	1.0938	0.0389	0.0421	0.0031	1.1018	1.0863	1.1176	1.0877	1.1159	1.0137	1.1900

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 10^{th} percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).
CV = Coefficient of variation.

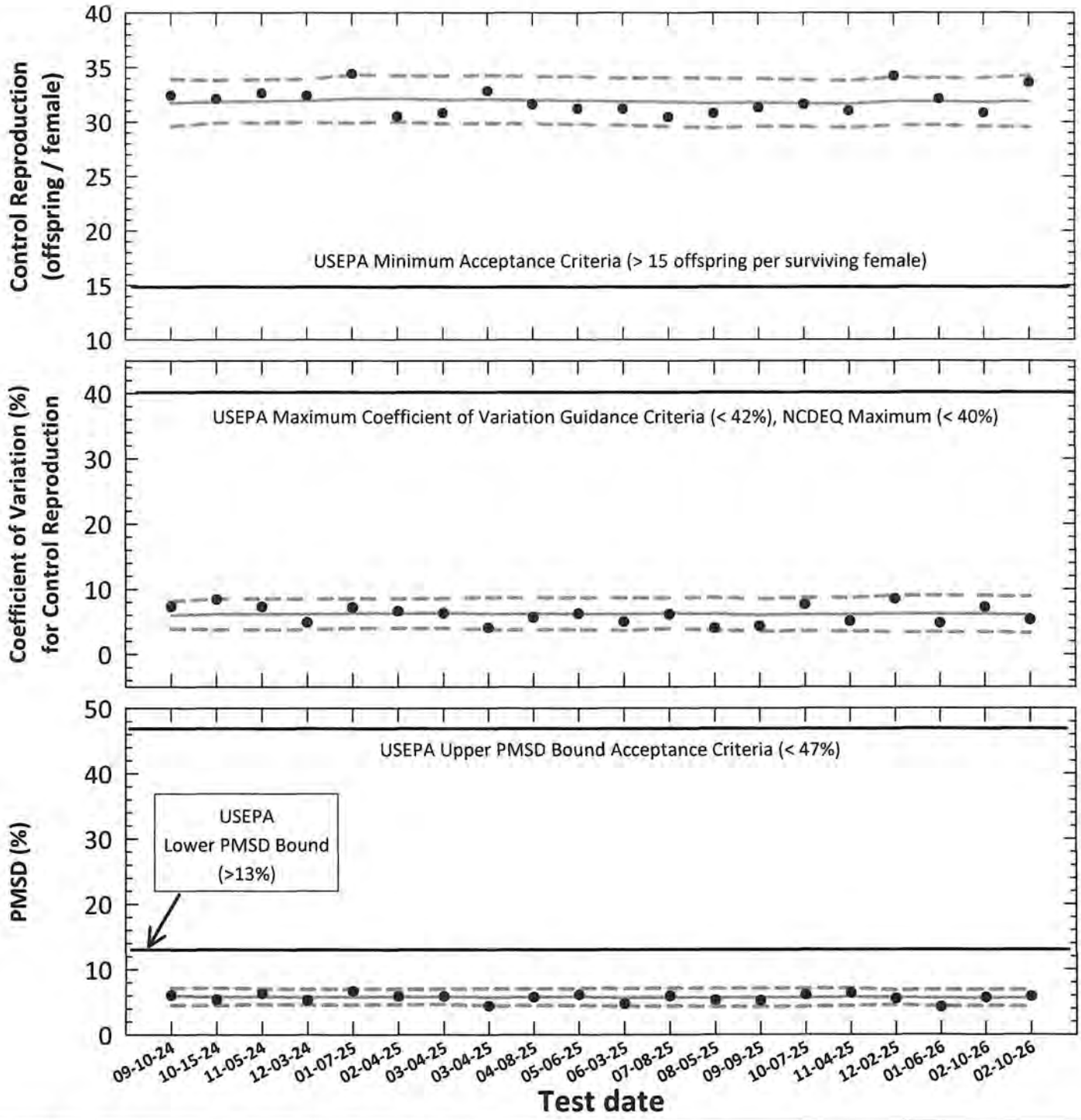
Entered and Reviewed by Jim Sumner

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 Mean (offspring/female)	Control Reproduction 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	09-10-24	100	32.4	7.3	1.947	6.0	31.7	29.6	33.9	6.0	3.9	8.1	5.8	4.5	7.2
2	10-15-24	100	32.1	8.4	1.715	5.3	31.9	29.9	33.8	6.1	3.8	8.5	5.8	4.5	7.2
3	11-05-24	100	32.6	7.3	2.050	6.3	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6	7.0
4	12-03-24	100	32.4	4.9	1.708	5.3	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
5	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
6	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
7	03-04-25	100	30.8	6.3	1.797	5.8	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0
8	03-04-25	100	32.8	4.0	1.430	4.4	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4	7.0
9	04-08-25	100	31.6	5.6	1.806	5.7	32.0	29.8	34.1	6.2	3.8	8.7	5.7	4.4	7.0
10	05-06-25	100	31.2	6.2	1.889	6.1	32.0	29.8	34.1	6.2	3.8	8.6	5.7	4.4	7.0
11	06-03-25	100	31.2	5.0	1.470	4.7	31.9	29.7	34.0	6.1	3.6	8.6	5.7	4.3	7.1
12	07-08-25	100	30.4	6.0	1.776	5.8	31.8	29.6	34.1	6.2	3.9	8.6	5.7	4.3	7.1
13	08-05-25	100	30.8	4.0	1.623	5.3	31.7	29.5	34.0	6.2	3.6	8.7	5.7	4.3	7.1
14	09-09-25	100	31.3	4.3	1.622	5.2	31.8	29.6	34.0	6.0	3.5	8.5	5.7	4.3	7.0
15	10-07-25	100	31.6	7.6	1.925	6.1	31.7	29.6	33.9	6.1	3.6	8.7	5.7	4.3	7.1
16	11-04-25	100	31.0	5.0	1.966	6.3	31.7	29.5	33.8	6.1	3.5	8.6	5.7	4.4	7.1
17	12-02-25	100	34.2	8.5	1.866	5.5	31.9	29.7	34.1	6.2	3.3	9.0	5.6	4.5	6.8
18	01-06-26	100	32.1	4.7	1.354	4.2	31.9	29.7	34.0	6.2	3.3	9.0	5.6	4.3	6.9
19	02-10-26	100	30.8	7.1	1.734	5.6	31.8	29.6	34.0	6.1	3.3	8.9	5.6	4.3	6.9
20	02-10-26	100	33.6	5.3	1.962	5.8	31.9	29.5	34.3	6.1	3.3	8.9	5.6	4.3	6.9

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 Summer

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 321 – New Algae/YWT

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 7451				
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:	02-10-26 0540 TO 0800									
Culture board:	02-05-26 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	2	6	7	11	13	17	18	19	22
Transfer vessel information:	pH (S.U.): 7.90 Temperature (°C): 25.1									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	ORANGE
Incubator number and shelf location:	2B2

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	02-10-26	0810	NEW 01-21-26	NEW 01-19-26	02-04-26 B	JH
1	02-11-26	0610	↓	↓	↓	JH
2	02-12-26	0615	↓	↓	02-09-26 A	JH
3	02-13-26	0610	↓	↓	↓	JH
4	02-14-26	0700	↓	↓	02-09-26 B	JK
5	02-15-26	0700	↓	↓	↓	JH
6	02-16-26	0625	↓	↓	↓	JA
7	02-17-26	0714				JK

*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-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	13066485

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:	33.6	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	5.3%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1093.8

Environmental Testing Solutions, Inc

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** **321 – New Algae/YWT**

CONTROL ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	4	4	4	4	5	3	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	13	13	12	11	13	13	11	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	19	17	19	18	15	18	16	16	15	17
Total young produced		35	33	36	35	31	34	32	34	31	35
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:	33.6

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	6	4	4	5	5	6	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	12	14	12	12	12	12	13	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	18	19	19	15	18	18	14	17	18
Total young produced		31	37	35	37	32	35	36	30	34	34
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	34.1
% Reduction from Control:	-1.5%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 321 – New Algae/YWT

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	5	5	6	4	4	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	14	12	12	13	11	14	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	17	17	14	15	15	18	15	19	17	16
Total young produced		35	31	33	32	33	35	30	38	34	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:	33.4
% 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	6	4	4	3	4	5	3	3	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	10	11	13	13	10	12	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	18	14	16	15	17	19	15	16	15
Total young produced		33	35	28	30	32	35	32	30	33	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.0
% Reduction from Control:	4.8%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 321 – New Algae/YWT

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.9
% Reduction from Control:	46.77.

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2.3
% Reduction from Control:	93.27.

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	4	4	4	4	5	3	5	5	5	43
5	12	12	13	13	12	11	13	13	11	13	123
6	0	0	0	0	0	0	0	0	0	0	0
7	19	17	19	18	15	18	16	16	15	17	170
Total	**35**	**33**	**36**	**35**	**31**	**34**	**32**	**34**	**31**	**35**	**336**

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	6	4	4	3	4	5	3	3	5	5	42
5	12	13	10	11	13	13	10	12	12	12	118
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	14	16	15	17	19	15	16	15	160
Total	**33**	**35**	**28**	**30**	**32**	**35**	**32**	**30**	**33**	**32**	**320**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	4	4	5	5	6	4	4	4	46
5	12	13	12	14	12	12	12	12	13	12	124
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	19	19	15	18	18	14	17	18	171
Total	**31**	**37**	**35**	**37**	**32**	**35**	**36**	**30**	**34**	**34**	**341**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	4	5	2	4	4	3	4	4	37
5	10	9	7	11	7	5	8	10	8	6	81
6	0	0	0	0	0	0	0	0	0	0	0
7	6	5	5	3	10	10	5	4	4	9	61
Total	**20**	**17**	**16**	**19**	**19**	**19**	**17**	**17**	**16**	**19**	**179**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	5	5	6	4	4	5	5	5	48
5	13	10	14	12	12	13	11	14	12	12	123
6	0	0	0	0	0	0	0	0	0	0	0
7	17	17	14	15	15	18	15	19	17	16	163
Total	**35**	**31**	**33**	**32**	**33**	**35**	**30**	**38**	**34**	**33**	**334**

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	1	2	3	3	1	1	1	1	17
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	3	0	0	0	2	1	0	0	6
Total	**2**	**2**	**4**	**2**	**3**	**3**	**3**	**2**	**1**	**1**	**23**

Entered and Reviewed by Jim Summey

ETS
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: 321, New algal slant + YWT consumbables

Test dates: February 10-17, 2026

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	35	33	36	35	31	34	32	34	31	35	100	33.6	5.3	Not applicable
600	31	37	35	37	32	35	36	30	34	34	100	34.1	7.1	-1.5
800	35	31	33	32	33	35	30	38	34	33	100	33.4	6.8	0.6
1000	33	35	28	30	32	35	32	30	33	32	100	32.0	6.9	4.8
1200	20	17	16	19	19	19	17	17	16	19	100	17.9	8.1	46.7
1400	2	2	4	2	3	3	3	2	1	1	100	2.3	41.2	93.2

Dunnett's MSD value: 1.962

PMSD: 5.8

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

Upper PMSD bound determined by USEPA (90^{th} 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

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	2/10/2026	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	2/17/2026	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	35.000	33.000	36.000	35.000	31.000	34.000	32.000	34.000	31.000	35.000
600	31.000	37.000	35.000	37.000	32.000	35.000	36.000	30.000	34.000	34.000
800	35.000	31.000	33.000	32.000	33.000	35.000	30.000	38.000	34.000	33.000
1000	33.000	35.000	28.000	30.000	32.000	35.000	32.000	30.000	33.000	32.000
1200	20.000	17.000	16.000	19.000	19.000	19.000	17.000	17.000	16.000	19.000
1400	2.000	2.000	4.000	2.000	3.000	3.000	3.000	2.000	1.000	1.000

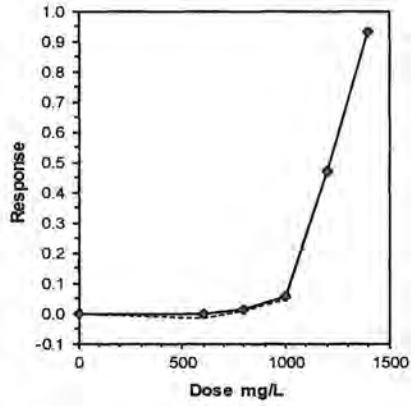
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	33.600	1.0000	33.600	31.000	36.000	5.287	10				33.850	1.0000
600	34.100	1.0149	34.100	30.000	37.000	7.110	10	-0.583	2.287	1.962	33.850	1.0000
800	33.400	0.9940	33.400	30.000	38.000	6.798	10	0.233	2.287	1.962	33.400	0.9867
1000	32.000	0.9524	32.000	28.000	35.000	6.910	10	1.865	2.287	1.962	32.000	0.9453
*1200	17.900	0.5327	17.900	16.000	20.000	8.096	10	18.301	2.287	1.962	17.900	0.5288
*1400	2.300	0.0685	2.300	1.000	4.000	41.247	10	36.486	2.287	1.962	2.300	0.0679

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.63075	1.035	-0.1202	-0.1067
Bartlett's Test indicates equal variances (p = 0.11)	8.91116	15.0863		

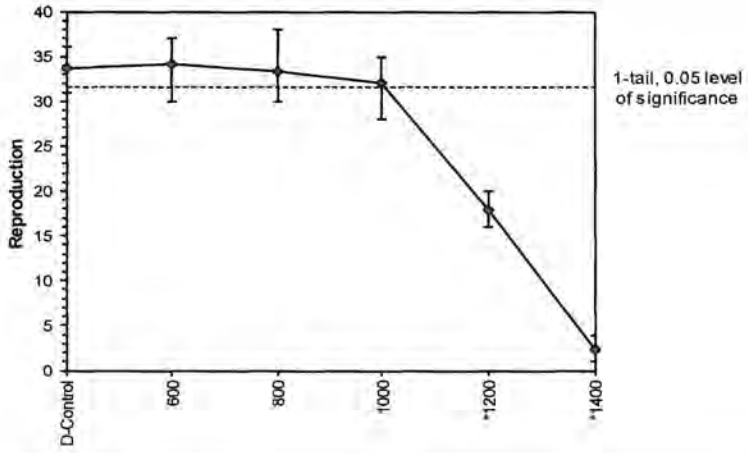
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	1200	1095.45		1.96164	0.05838	1680.43	3.67963	7.2E-43	5, 54

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	977.5	68.8428	774.792	1013.4	-0.9343
IC10	1021.77	13.5426	980.517	1036.44	-1.8934
IC15	1045.78	9.49067	1023.29	1058.44	-0.2992
IC20	1069.79	8.43577	1050.45	1082.72	-0.1849
IC25	1093.79	7.55197	1077.2	1106.95	-0.0379
IC40	1165.82	6.58231	1152.35	1179.72	0.3214
IC50	1212.5	6.13577	1199.33	1223.9	-0.0003



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** **321 – New Algae/YWT**

Daily Chemistry:

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

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.82	7.88	7.78	7.92	7.83	7.95
	Dissolved oxygen (mg/L)	8.4	8.0	8.4	8.2	8.5	8.1
	Conductivity (µmhos/cm)	295		302		295	
	Alkalinity (mg $CaCO_3$/L)	63				60	
	Hardness (mg $CaCO_3$/L)	86				84	
	Temperature (°C)	24.8	25.1	24.9	25.3	24.8	24.7
600 mg NaCl/L	pH (S.U.)	7.92	7.87	7.84	7.95	7.89	7.96
	Dissolved oxygen (mg/L)	8.5	8.0	8.3	8.3	8.5	8.1
	Conductivity (µmhos/cm)	1380		1380		1370	
	Temperature (°C)	24.9	24.9	24.8	25.1	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.93	7.87	7.85	7.96	7.89	7.96
	Dissolved oxygen (mg/L)	8.5	8.0	8.2	8.2	8.5	8.2
	Conductivity (µmhos/cm)	1720		1750		1740	
	Temperature (°C)	25.0	24.9	24.8	25.2	24.9	24.8
1000 mg NaCl/L	pH (S.U.)	7.94	7.87	~~7.88~~ 7.85 recalibrated	7.96	7.91	7.97
	Dissolved oxygen (mg/L)	8.6	8.0	8.2	8.2	8.5	8.1
	Conductivity (µmhos/cm)	2060		2080		2050	
	Temperature (°C)	24.9	25.1	24.7	25.2	25.0	24.8
1200 mg NaCl/L	pH (S.U.)	7.95	7.87	7.88	7.97	7.92	7.97
	Dissolved oxygen (mg/L)	8.5	8.0	8.2	8.3	8.5	8.1
	Conductivity (µmhos/cm)	2400		2420		2440	
	Temperature (°C)	24.9	25.1	24.9	25.2	25.0	24.8
1400 mg NaCl/L	pH (S.U.)	7.95	7.88	7.88	7.97	7.92	7.97
	Dissolved oxygen (mg/L)	8.4	8.0	8.2	8.3	8.5	8.2
	Conductivity (µmhos/cm)	2760		2770		2770	
	Temperature (°C)	24.9	25.1	24.9	25.2	25.0	24.8
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

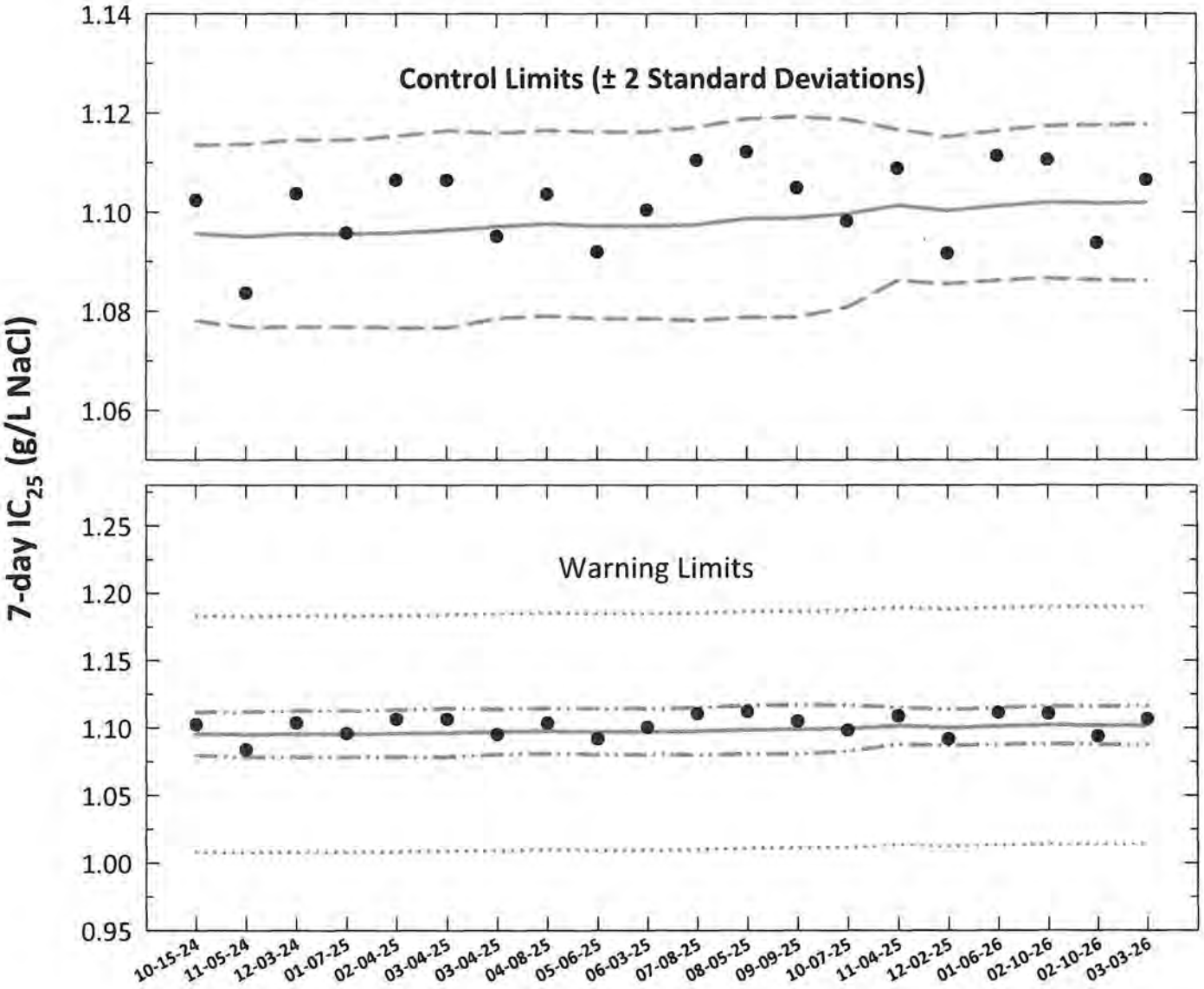
Species: ***Ceriodaphnia dubia***

CdNaClCR #: 321 – New Algae/YWT

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.85	8.04	~~7.85~~ 7.79	8.01	7.81	7.98	7.87	8.09
	Dissolved oxygen (mg/L)	8.5	8.2	8.6	8.1	8.6	8.2	8.4	7.6
	Conductivity (µmhos/cm)	297		285		277		306	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)			88					
	Temperature (°C)	24.9	25.0	24.8	25.1	24.8	25.3	24.9	25.3
600 mg NaCl/L	pH (S.U.)	7.89	8.03	7.81	8.03	7.86	7.97	7.91	8.06
	Dissolved oxygen (mg/L)	8.4	8.2	8.6	8.0	8.6	8.2	8.4	7.6
	Conductivity (µmhos/cm)	1360		1360		1340		1430	
	Temperature (°C)	25.0	25.3	24.9	25.0	24.8	25.0	24.7	25.1
800 mg NaCl/L	pH (S.U.)	7.90	8.03	7.94	8.02	7.93	7.99	7.94	8.05
	Dissolved oxygen (mg/L)	8.5	8.3	8.5	8.1	8.5	8.3	8.4	7.6
	Conductivity (µmhos/cm)	1720		1690		1650		1790	
	Temperature (°C)	25.0	25.1	24.9	25.2	24.9	25.0	24.9	25.2
1000 mg NaCl/L	pH (S.U.)	7.90	8.03	7.94	8.03	7.98	7.99	7.94	8.05
	Dissolved oxygen (mg/L)	8.4	8.3	8.6	8.1	8.5	8.2	8.4	7.6
	Conductivity (µmhos/cm)	2040		2030		1990		2110	
	Temperature (°C)	24.9	24.9	24.8	25.2	24.8	24.9	24.8	25.2
1200 mg NaCl/L	pH (S.U.)	7.91	8.02	7.97	8.02	8.00	7.99	7.96	8.03
	Dissolved oxygen (mg/L)	8.4	8.3	8.5	8.1	8.5	8.3	8.3	7.6
	Conductivity (µmhos/cm)	2400		(2340) ~~2360~~		2310		2440	
	Temperature (°C)	24.9	24.9	24.8	25.3	24.8	24.9	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	7.92	8.01	7.98	8.01	8.01	7.98	7.98	8.02
	Dissolved oxygen (mg/L)	8.4	8.3	8.5	8.1	8.4	8.2	8.3	7.6
	Conductivity (µmhos/cm)	2730		2670		2640		2790	
	Temperature (°C)	24.9	25.2	24.8	25.0	24.8	25.1	25.0	25.4
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

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	10-15-24	1.1024	0.0423	0.0397	0.0035	1.0956	1.0781	1.1135	1.0796	1.1116	1.0080	1.1833
2	11-05-24	1.0837	0.0349	0.0394	0.0037	1.0951	1.0767	1.1137	1.0783	1.1118	1.0075	1.1827
3	12-03-24	1.1037	0.0428	0.0396	0.0037	1.0956	1.0769	1.1146	1.0785	1.1126	1.0079	1.1832
4	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
5	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
6	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841
7	03-04-25	1.0951	0.0394	0.0402	0.0037	1.0971	1.0786	1.1159	1.0802	1.1139	1.0093	1.1848
8	04-08-25	1.1036	0.0428	0.0405	0.0037	1.0976	1.0791	1.1165	1.0807	1.1145	1.0098	1.1854
9	05-06-25	1.0920	0.0382	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
10	06-03-25	1.1004	0.0416	0.0403	0.0037	1.0972	1.0785	1.1162	1.0802	1.1142	1.0094	1.1850
11	07-08-25	1.1104	0.0455	0.0404	0.0038	1.0975	1.0782	1.1171	1.0799	1.1151	1.0097	1.1853
12	08-05-25	1.1121	0.0462	0.0409	0.0040	1.0987	1.0788	1.1189	1.0806	1.1167	1.0108	1.1866
13	09-09-25	1.1049	0.0433	0.0410	0.0040	1.0989	1.0789	1.1193	1.0807	1.1171	1.0110	1.1868
14	10-07-25	1.0982	0.0407	0.0412	0.0037	1.0996	1.0808	1.1187	1.0825	1.1167	1.0116	1.1876
15	11-04-25	1.1088	0.0448	0.0419	0.0030	1.1013	1.0862	1.1166	1.0876	1.1150	1.0132	1.1894
16	12-02-25	1.0917	0.0381	0.0415	0.0029	1.1003	1.0856	1.1152	1.0869	1.1136	1.0123	1.1883
17	01-06-26	1.1113	0.0458	0.0419	0.0030	1.1012	1.0863	1.1164	1.0877	1.1148	1.0131	1.1893
18	02-10-26	1.1106	0.0455	0.0422	0.0030	1.1020	1.0867	1.1175	1.0881	1.1158	1.0138	1.1901
19	02-10-26	1.0938	0.0389	0.0421	0.0031	1.1018	1.0863	1.1176	1.0877	1.1159	1.0137	1.1900
20	03-03-26	1.1065	0.0439	0.0421	0.0031	1.1019	1.0863	1.1177	1.0877	1.1160	1.0137	1.1900

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 $IC_{25} \pm 2$ standard deviations converted to anti-logarithmic values.

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

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

CV = Coefficient of variation.

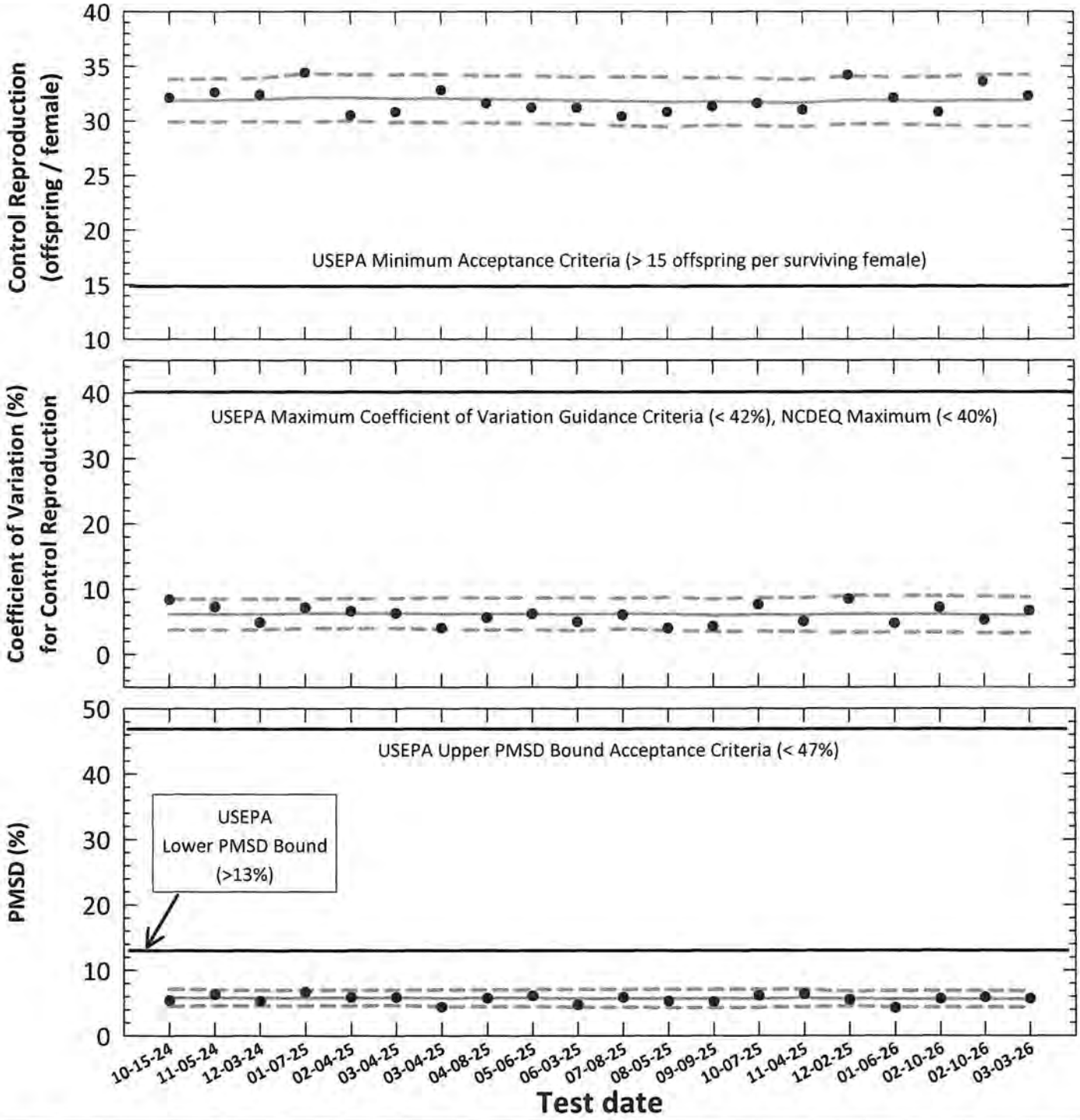
Entered and Reviewed by Jim Sumner

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	Control Reproduction		Test										
		Survival	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	10-15-24	100	32.1	8.4	1.715	5.3	31.9	29.9	33.8	6.1	3.8	8.5	5.8	4.5	7.2
2	11-05-24	100	32.6	7.3	2.050	6.3	31.9	29.9	33.9	6.1	3.8	8.5	5.8	4.6	7.0
3	12-03-24	100	32.4	4.9	1.708	5.3	31.9	30.0	33.9	6.1	3.8	8.5	5.7	4.5	6.9
4	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
5	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
6	03-04-25	100	30.8	6.3	1.797	5.8	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0
7	03-04-25	100	32.8	4.0	1.430	4.4	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4	7.0
8	04-08-25	100	31.6	5.6	1.806	5.7	32.0	29.8	34.1	6.2	3.8	8.7	5.7	4.4	7.0
9	05-06-25	100	31.2	6.2	1.889	6.1	32.0	29.8	34.1	6.2	3.8	8.6	5.7	4.4	7.0
10	06-03-25	100	31.2	5.0	1.470	4.7	31.9	29.7	34.0	6.1	3.6	8.6	5.7	4.3	7.1
11	07-08-25	100	30.4	6.0	1.776	5.8	31.8	29.6	34.1	6.2	3.9	8.6	5.7	4.3	7.1
12	08-05-25	100	30.8	4.0	1.623	5.3	31.7	29.5	34.0	6.2	3.6	8.7	5.7	4.3	7.1
13	09-09-25	100	31.3	4.3	1.622	5.2	31.8	29.6	34.0	6.0	3.5	8.5	5.7	4.3	7.0
14	10-07-25	100	31.6	7.6	1.925	6.1	31.7	29.6	33.9	6.1	3.6	8.7	5.7	4.3	7.1
15	11-04-25	100	31.0	5.0	1.966	6.3	31.7	29.5	33.8	6.1	3.5	8.6	5.7	4.4	7.1
16	12-02-25	100	34.2	8.5	1.866	5.5	31.9	29.7	34.1	6.2	3.3	9.0	5.6	4.5	6.8
17	01-06-26	100	32.1	4.7	1.354	4.2	31.9	29.7	34.0	6.2	3.3	9.0	5.6	4.3	6.9
18	02-10-26	100	30.8	7.1	1.734	5.6	31.8	29.6	34.0	6.1	3.3	8.9	5.6	4.3	6.9
19	02-10-26	100	33.6	5.3	1.962	5.8	31.9	29.5	34.3	6.1	3.3	8.9	5.6	4.3	6.9
20	03-03-26	100	32.3	6.7	1.823	5.6	31.9	29.5	34.2	6.0	3.3	8.8	5.6	4.3	6.8

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 Summey

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 322

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 24166				
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:	03-03-26 0530 TO 0630									
Culture board:	02-24-26 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	2	3	5	8	12	13	16	17	18	21
Transfer vessel information:	pH (S.U.): 8.18 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	BWC
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	03-03-26	0845	02-26-26	02-26-26	02-25-26A	JK
1	03-04-26	0650	↓	↓	↓	JK
2	03-05-26	0650			02-25-26B	JK
3	03-06-26	0645 12+3 04-02-26 JK			↓	JK
4	03-07-26	0645			03-05-26A	JK
5	03-08-26	0708			↓	JK
6	03-09-26	0810	↓	↓	↓	JK
7	03-10-26	0646				JK

*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-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2560B-2010	Digital Thermometer	1506648S

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:	32.3	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	6.7%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1106.5

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 322

CONTROL ***Survival and Reproduction Data***

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	4	4	5	5	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	13	12	12	13	12	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	16	16	17	15	18	17	18	15	15
Total young produced		31	30	33	33	32	36	33	35	29	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:	32.3

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	6	4	4	4	5	5	4	4	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	11	12	12	13	12	13	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	16	16	15	18	15	15	19	13	16	15
Total young produced		35	31	30	34	32	33	35	30	34	29
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.3
% Reduction from Control:	0.0%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** 322

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	3	5	4	4	4	4	5	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	12	9	12	12	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	15	14	16	17	16	14	15	19	17	16
Total young produced		30	29	34	30	32	30	32	34	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:	31.4
% Reduction from Control:	2.8%

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

Day		Replicate number									
		1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	3	4	4	3	3	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	11	10	12	10	12	12	11	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	15	19	16	17	15	18	16	16	13
Total young produced		30	30	32	34	31	30	33	32	33	27
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.2
% Reduction from Control:	3.4%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 322**

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	2	3	4	4	3	3	3	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	8	10	7	9	11	5	10	8	8
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	6	5	5	8	7	3	11	6	6	5
Total young produced		19	15	18	19	20	17	19	19	18	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:	18.1
% Reduction from Control:	44.0%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2.5
% Reduction from Control:	92.3%

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	5	4	4	4	5	5	4	4	4	4	43
5	11	10	13	12	12	13	12	13	10	12	118
6	0	0	0	0	0	0	0	0	0	0	0
7	15	16	16	17	15	18	17	18	15	15	162
Total	**31**	**30**	**33**	**33**	**32**	**36**	**33**	**35**	**29**	**31**	**323**

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	4	4	5	5	4	4	5	4	45
5	13	11	11	12	12	13	12	13	13	10	120
6	0	0	0	0	0	0	0	0	0	0	0
7	16	16	15	18	15	15	19	13	16	15	158
Total	**35**	**31**	**30**	**34**	**32**	**33**	**35**	**30**	**34**	**29**	**323**

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	3	5	4	4	4	4	5	5	4	4	42
5	12	10	12	9	12	12	12	10	10	12	111
6	0	0	0	0	0	0	0	0	0	0	0
7	15	14	18	17	16	14	15	19	17	16	161
Total	**30**	**29**	**34**	**30**	**32**	**30**	**32**	**34**	**31**	**32**	**314**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	3	4	4	3	3	5	5	4	40
5	10	11	10	12	10	12	12	11	12	10	110
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	19	18	17	15	18	16	16	13	162
Total	**30**	**30**	**32**	**34**	**31**	**30**	**33**	**32**	**33**	**27**	**312**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	2	3	4	4	3	3	3	4	4	33
5	10	8	10	7	9	11	5	10	8	8	86
6	0	0	0	0	0	0	0	0	0	0	0
7	6	5	5	8	7	3	11	6	6	5	62
Total	**19**	**15**	**18**	**19**	**20**	**17**	**19**	**19**	**18**	**17**	**181**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	3	1	2	2	3	1	3	3	21
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	2	0	1	0	0	0	1	0	0	4
Total	**2**	**3**	**3**	**2**	**2**	**2**	**3**	**2**	**3**	**3**	**25**

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: 322

Test dates: March 03-10, 2026

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	30	33	33	32	36	33	35	29	31	100	32.3	6.7	Not applicable
600	35	31	30	34	32	33	35	30	34	29	100	32.3	6.9	0.0
800	30	29	34	30	32	30	32	34	31	32	100	31.4	5.5	2.8
1000	30	30	32	34	31	30	33	32	33	27	100	31.2	6.6	3.4
1200	19	15	18	19	20	17	19	19	18	17	100	18.1	8.0	44.0
1400	2	3	3	2	2	2	3	2	3	3	100	2.5	21.1	92.3

Dunnett's MSD value: 1.823

PMSD: 5.6

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

Upper PMSD bound determined by USEPA (90^{th} 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 Jimi Summer

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Cumner

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	3/3/2026	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/10/2026	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	30.000	33.000	33.000	32.000	36.000	33.000	35.000	29.000	31.000
600	35.000	31.000	30.000	34.000	32.000	33.000	35.000	30.000	34.000	29.000
800	30.000	29.000	34.000	30.000	32.000	30.000	32.000	34.000	31.000	32.000
1000	30.000	30.000	32.000	34.000	31.000	30.000	33.000	32.000	33.000	27.000
1200	19.000	15.000	18.000	19.000	20.000	17.000	19.000	19.000	18.000	17.000
1400	2.000	3.000	3.000	2.000	2.000	2.000	3.000	2.000	3.000	3.000

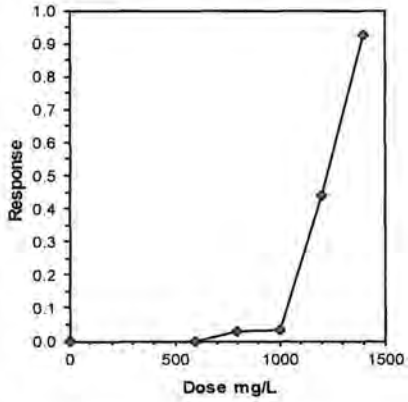
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	32.300	1.0000	32.300	29.000	36.000	6.696	10			32.300	1.0000
600	32.300	1.0000	32.300	29.000	35.000	6.853	10	105.50	75.00	32.300	1.0000
800	31.400	0.9721	31.400	29.000	34.000	5.454	10	92.50	75.00	31.400	0.9721
1000	31.200	0.9659	31.200	27.000	34.000	6.551	10	92.50	75.00	31.200	0.9659
*1200	18.100	0.5604	18.100	15.000	20.000	8.006	10	55.00	75.00	18.100	0.5604
*1400	2.500	0.0774	2.500	2.000	3.000	21.082	10	55.00	75.00	2.500	0.0774

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.90468	1.035	-0.1688	-0.1798
Bartlett's Test indicates unequal variances (p = 7.26E-03)	15.8578	15.0863		

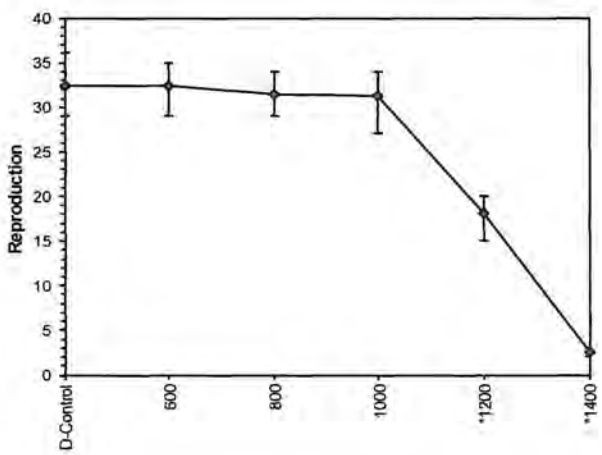
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1000	1200	1095.45	
Treatments vs D-Control				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1007.86	111.741	689.749	1020.99	-1.3157
IC10	1032.52	14.9269	1003.71	1044.43	-5.0599
IC15	1057.18	9.44554	1031.23	1068.09	-0.5883
IC20	1081.83	8.79005	1059.92	1091.78	-0.4754
IC25	1106.49	8.2968	1086.74	1116.54	-0.3736
IC40	1180.46	8.03883	1161.93	1192.24	-0.2118
IC50	1225	6.16838	1209.68	1233.36	-0.4347



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Used for PMSD calculation only.

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	3/3/2026	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	3/10/2026	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	30.000	33.000	33.000	32.000	36.000	33.000	35.000	29.000	31.000
600	35.000	31.000	30.000	34.000	32.000	33.000	35.000	30.000	34.000	29.000
800	30.000	29.000	34.000	30.000	32.000	30.000	32.000	34.000	31.000	32.000
1000	30.000	30.000	32.000	34.000	31.000	30.000	33.000	32.000	33.000	27.000
1200	19.000	15.000	18.000	19.000	20.000	17.000	19.000	19.000	18.000	17.000
1400	2.000	3.000	3.000	2.000	2.000	2.000	3.000	2.000	3.000	3.000

			Transform: Untransformed						1-Tailed	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	32.300	1.0000	32.300	29.000	36.000	6.696	10			
600	32.300	1.0000	32.300	29.000	35.000	6.853	10	0.000	2.287	1.823
800	31.400	0.9721	31.400	29.000	34.000	5.454	10	1.129	2.287	1.823
1000	31.200	0.9659	31.200	27.000	34.000	6.551	10	1.380	2.287	1.823
*1200	18.100	0.5604	18.100	15.000	20.000	8.006	10	17.812	2.287	1.823
*1400	2.500	0.0774	2.500	2.000	3.000	21.082	10	37.380	2.287	1.823

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.90468	1.035	-0.1688	-0.1798
Bartlett's Test indicates unequal variances (p = 7.26E-03)	15.8578	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	1200	1095.45		1.82297	0.05644	1478.07	3.17778	4.4E-43	5, 54

Environmental Testing Solutions, Inc.

Species: *Ceriodaphnia dubia* **CdNaClCR #: 322**

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	N	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.16	7.85	7.76	7.80	7.80	7.87
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	293		306		298	
	Alkalinity (mg $CaCO_3$/L)	63				62	
	Hardness (mg $CaCO_3$/L)	86			8.03 10-26-21	88	
	Temperature (°C)	24.9	25.3	25.0	25.2	24.9	24.9
600 mg NaCl/L	pH (S.U.)	8.14	7.85	7.81	7.87	7.85	7.89
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	1300		1360		1360	
	Temperature (°C)	25.0	25.0	25.0	24.9	24.9	25.1
800 mg NaCl/L	pH (S.U.)	8.13	7.86	7.84	7.87	7.86	7.89
	Dissolved oxygen (mg/L)	8.2	8.2	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	1650		1740		1770	
	Temperature (°C)	25.0	25.0	24.9	24.9	25.0	24.8
1000 mg NaCl/L	pH (S.U.)	8.12	7.85	7.84	7.89	7.87	7.90
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	1960		2120		2100	
	Temperature (°C)	25.0	25.2	24.9	25.1	24.8	24.8
1200 mg NaCl/L	pH (S.U.)	8.11	7.86	7.85	7.89	7.89	7.90
	Dissolved oxygen (mg/L)	8.2	8.2	8.4	8.2	8.4	8.1
	Conductivity (µmhos/cm)	2300		2500		2490	
	Temperature (°C)	25.0	25.2	25.0	25.1	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	8.10	7.86	7.86	7.89	7.89	7.93
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.2	8.4	8.0
	Conductivity (µmhos/cm)	2620		2800		2810	
	Temperature (°C)	25.1	25.1	25.0	25.1	24.8	24.8
		Initial	Final	Initial	Final	Initial	Final

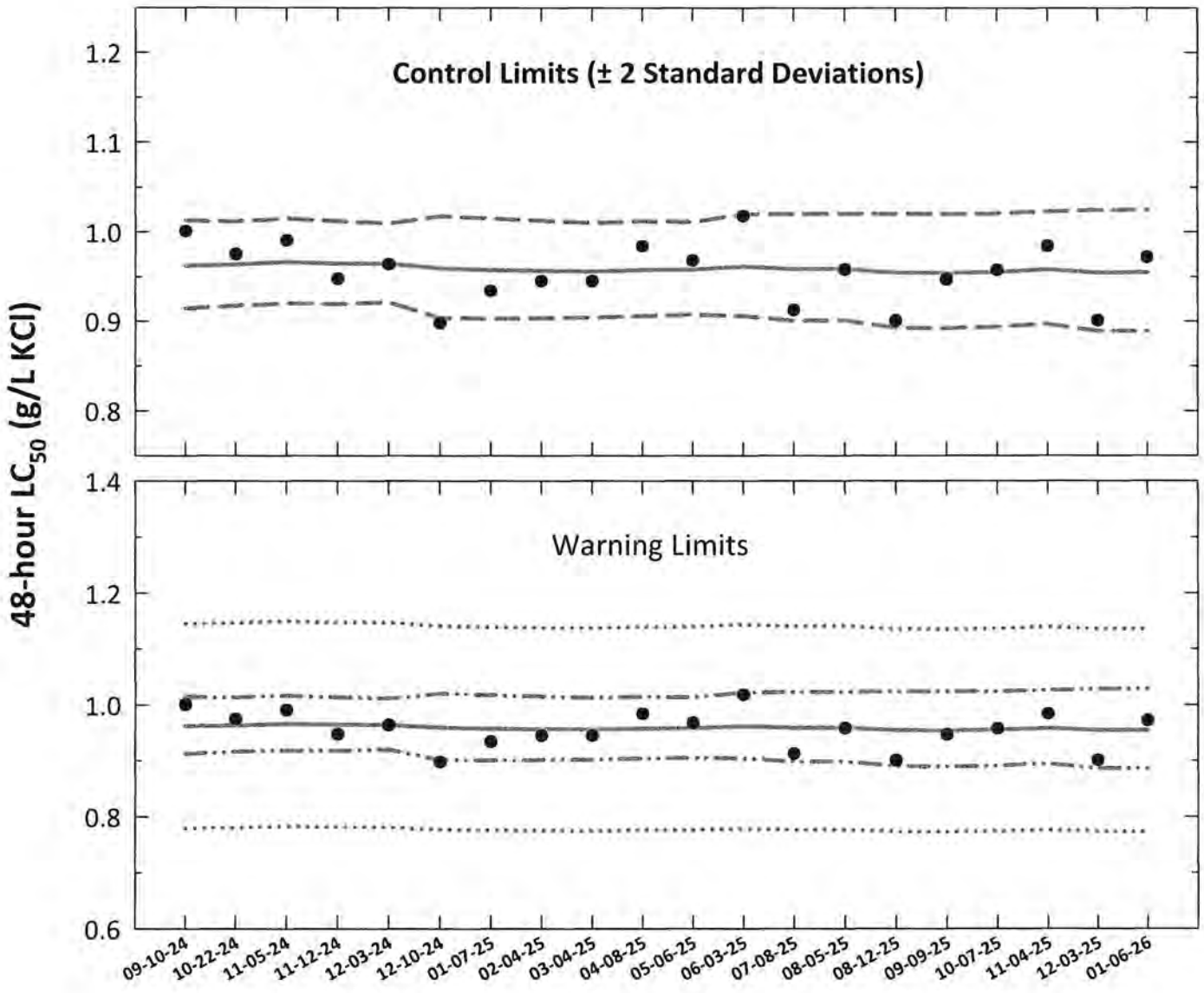
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia***

CdNaClCR #: 322

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.79	7.91	7.68	8.05	7.77	7.94	7.85	8.12
	Dissolved oxygen (mg/L)	8.4	8.2	8.6	8.1	8.5	8.2	8.4	7.7
	Conductivity (µmhos/cm)	302		~~278~~ 290		281		298	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.8	24.9	24.8	25.1	24.8	25.2	25.0	25.3
600 mg NaCl/L	pH (S.U.)	7.86	7.94	7.71	8.04	7.79	7.95	7.90	8.07
	Dissolved oxygen (mg/L)	8.4	8.3	8.6	8.1	8.4	8.2	8.4	7.7
	Conductivity (µmhos/cm)	1370		1290		1330		1350	
	Temperature (°C)	24.9	25.2	24.8	24.9	24.9	25.2	24.8	25.1
800 mg NaCl/L	pH (S.U.)	7.88	7.95	7.84	8.03	7.95	7.96	7.92	8.06
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.1	8.4	8.2	8.4	7.7
	Conductivity (µmhos/cm)	1750		1660		1710		1770	
	Temperature (°C)	25.0	24.9	24.8	24.9	24.9	24.9	24.8	25.3
1000 mg NaCl/L	pH (S.U.)	7.89	7.95	7.84	8.02	7.98	7.97	7.94	8.06
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.1	8.4	8.2	8.4	7.7
	Conductivity (µmhos/cm)	2080		2020		2060		2130	
	Temperature (°C)	25.0	24.9	24.9	24.9	25.0	25.1	24.9	25.4
1200 mg NaCl/L	pH (S.U.)	7.91	7.96	7.88	8.02	7.99	7.98	7.95	8.04
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.0	8.4	8.2	8.4	7.7
	Conductivity (µmhos/cm)	2460		2360 ~~2020~~		2430		2480	
	Temperature (°C)	24.9	25.1	24.9	25.1	25.0	24.9	24.9	25.2
1400 mg NaCl/L	pH (S.U.)	7.91	7.96	7.89	8.01	8.00	7.98	7.96	8.04
	Dissolved oxygen (mg/L)	8.4	8.2	8.5	8.1	8.5	8.2	8.4	7.7
	Conductivity (µmhos/cm)	2830		2660		2770		2820	
	Temperature (°C)	24.9	24.9	24.8	25.1	25.0	24.9	24.9	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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	09-10-24	1.0007	0.0003	-0.0168	0.0111	0.9621	0.9141	1.0127	0.9122	1.0147	0.7793	1.1449
2	10-22-24	0.9751	-0.0109	-0.0161	0.0106	0.9635	0.9177	1.0117	0.9160	1.0135	0.7805	1.1466
3	11-05-24	0.9904	-0.0042	-0.0149	0.0107	0.9662	0.9199	1.0148	0.9183	1.0165	0.7826	1.1498
4	11-12-24	0.9473	-0.0235	-0.0157	0.0104	0.9645	0.9192	1.0120	0.9175	1.0137	0.7812	1.1477
5	12-03-24	0.9637	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
6	12-10-24	0.8977	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
7	01-07-25	0.9340	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
8	02-04-25	0.9448	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
9	03-04-25	0.9448	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
10	04-08-25	0.9839	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
11	05-06-25	0.9680	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
12	06-03-25	1.0177	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
13	07-08-25	0.9124	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
14	08-05-25	0.9576	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
15	08-12-25	0.9003	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
16	09-09-25	0.9465	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351
17	10-07-25	0.9572	-0.0190	-0.0200	0.0144	0.9549	0.8935	1.0205	0.8907	1.0236	0.7735	1.1363
18	11-04-25	0.9840	-0.0070	-0.0187	0.0143	0.9579	0.8967	1.0231	0.8940	1.0260	0.7759	1.1399
19	12-03-25	0.9007	-0.0454	-0.0204	0.0154	0.9542	0.8888	1.0243	0.8857	1.0277	0.7729	1.1355
20	01-06-26	0.9716	-0.0125	-0.0203	0.0154	0.9544	0.8888	1.0247	0.8857	1.0281	0.7730	1.1357

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

ETC

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 # 187

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 K stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2447

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.84	7.92	7.87
	Dissolved oxygen (mg/L)	8.5	8.1	8.1
	Conductivity (µmhos/cm)	289		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	90		
	Temperature (°C)	24.3	25.2	25.1
500 mg/L	pH (S.U.)	7.98	7.90	7.86
	Dissolved oxygen (mg/L)	8.5	8.1	8.2
	Conductivity (µmhos/cm)	1100		
	Temperature (°C)	24.1	25.3	25.0
750 mg/L	pH (S.U.)	7.99	7.91	7.87
	Dissolved oxygen (mg/L)	8.5	8.1	8.2
	Conductivity (µmhos/cm)	1470		
	Temperature (°C)	24.6	25.3	25.0
1000 mg/L	pH (S.U.)	7.99	7.92	7.87
	Dissolved oxygen (mg/L)	8.4	8.1	8.2
	Conductivity (µmhos/cm)	1860		
	Temperature (°C)	24.3	25.0	24.9
1250 mg/L	pH (S.U.)	8.00	7.92	7.88
	Dissolved oxygen (mg/L)	8.4	8.1	8.1
	Conductivity (µmhos/cm)	2250		
	Temperature (°C)	24.8	25.2	25.2
1500 mg/L	pH (S.U.)	8.00	7.92	
	Dissolved oxygen (mg/L)	8.4	8.1	
	Conductivity (µmhos/cm)	2680		
	Temperature (°C)	24.5	25.2	

01.06.26

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurem 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 t 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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN2501000503C
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664

ETC

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 # 187

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	01-06-26	*0605+ 0900	JP	1127	JP	7D	Bright P.1K	12-30-25A
24	01-07-26			1129	JP			
48 Termination	01-08-26			1135	JP			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	12-25-25
Age (1 to 14 days old):	5 to 6 days
Hatch date and times:	12-31-25 1141 am 01-01-26 0640
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.21
	Temperature (°C): 24.7°C

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

Survival Data **(number of living organisms):**

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	5 sd	10	7 3d	8 2d	0 10d	1 9d 2D 01-07-26	0 10d	0 10d
48 Termination	10	10	10	10	5	10	7	8	0	1	0	0
Mean Survival	100%		100%		75%		75%		5%		0%	

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

Statistics:

Method	SK
Lower 95% confidence limit (mg KCl/L)	891.8
Upper 95% confidence limit (mg KCl/L)	1058.5
48-hour LC_{50} (mg KCl/L)	971.6

Comments:

Test Reviewed by: JH

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	1/6/2026	Test ID:	PpKClAC	Sample ID:	PPKCLAC
End Date:	1/8/2026	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	0.5000	1.0000
1000	0.7000	0.8000
1250	0.0000	0.1000
1500	0.0000	0.0000

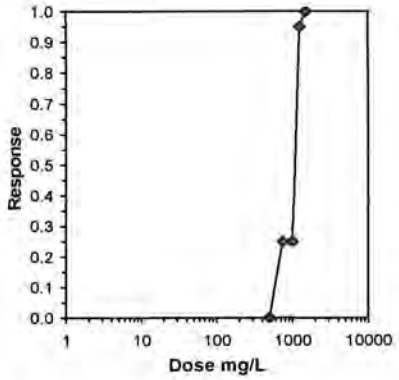
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.5928	0	20
750	0.7500	0.7500	1.0987	0.7854	1.4120	40.328	2	1.506	2.850	0.5928	5	20
1000	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	1.744	2.850	0.5928	5	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	5.633	2.850	0.5928	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				

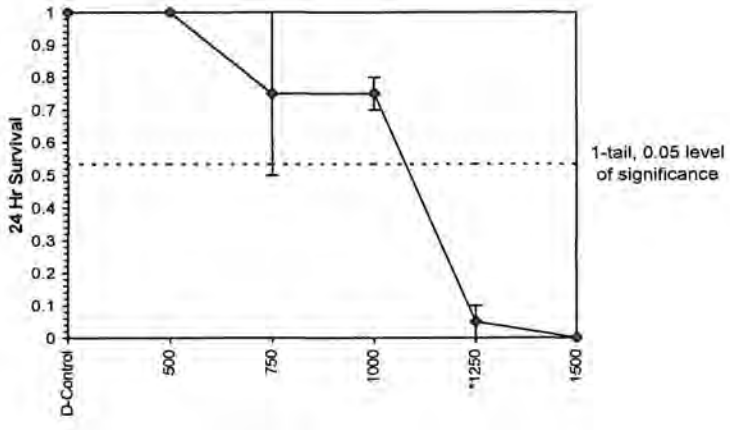
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.44122	0.45254	0.45993	0.04327	0.01162	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	971.622	891.84	1058.54
5.0%	986.894	900.123	1082.03
10.0%	1007.46	912.848	1111.87
20.0%	1054.44	947.001	1174.06
Auto-0.0%	971.622	891.84	1058.54



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	1/6/2026	Test ID:	PpKClAC	Sample ID:	PPKCLAC
End Date:	1/8/2026	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	0.5000	1.0000
1000	0.7000	0.8000
1250	0.0000	0.1000
1500	0.0000	0.0000

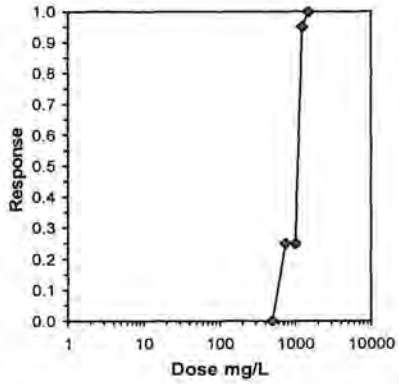
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.5928	0	20
750	0.7500	0.7500	1.0987	0.7854	1.4120	40.328	2	1.506	2.850	0.5928	5	20
1000	0.7500	0.7500	1.0492	0.9912	1.1071	7.818	2	1.744	2.850	0.5928	5	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	5.633	2.850	0.5928	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				

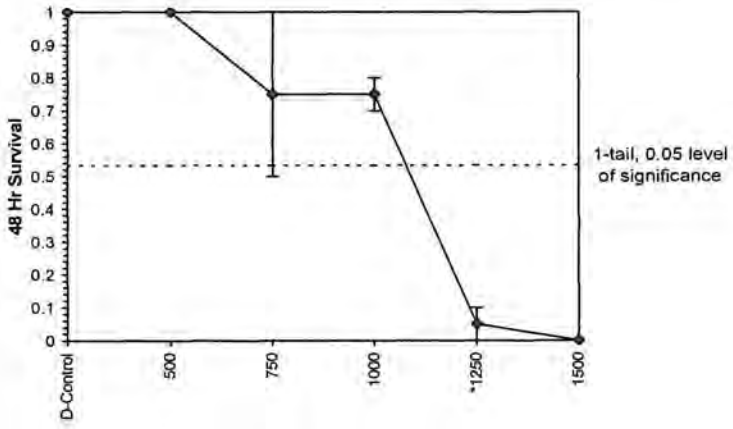
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.44122	0.45254	0.45993	0.04327	0.01162	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

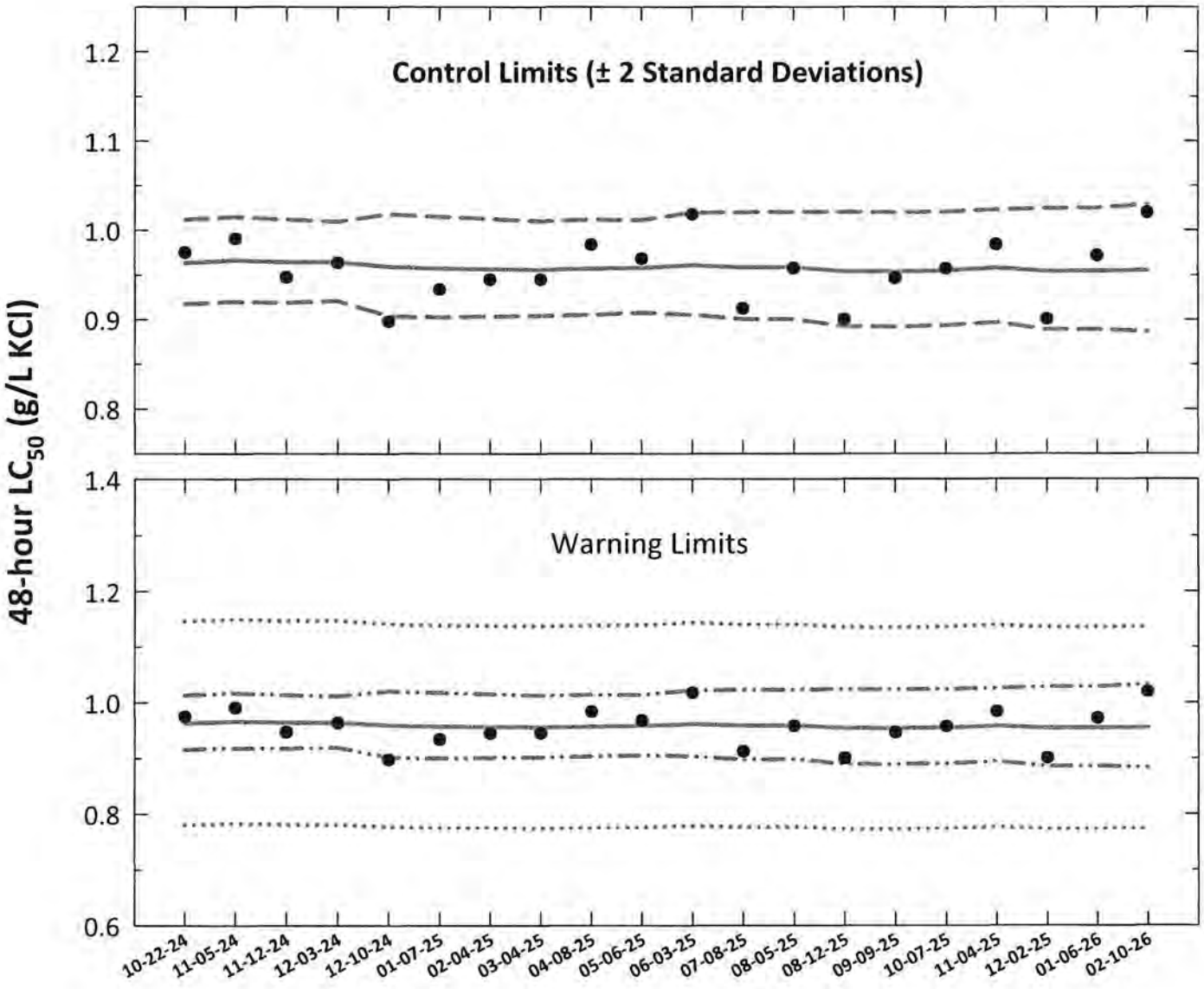
Trim Level	EC50	95% CL	
0.0%	971.622	891.84	1058.54
5.0%	986.894	900.123	1082.03
10.0%	1007.46	912.848	1111.87
20.0%	1054.44	947.001	1174.06
Auto-0.0%	971.622	891.84	1058.54



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} ± $S_{A.75}$ 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	**10-22-24**	**0.9751**	-0.0109	-0.0161	0.0106	0.9635	0.9177	1.0117	0.9160	1.0135	0.7805	1.1466
2	**11-05-24**	**0.9904**	-0.0042	-0.0149	0.0107	0.9662	0.9199	1.0148	0.9183	1.0165	0.7826	1.1498
3	**11-12-24**	**0.9473**	-0.0235	-0.0157	0.0104	0.9645	0.9192	1.0120	0.9175	1.0137	0.7812	1.1477
4	**12-03-24**	**0.9637**	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
5	**12-10-24**	**0.8977**	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
6	**01-07-25**	**0.9340**	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
7	**02-04-25**	**0.9448**	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
8	**03-04-25**	**0.9448**	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
9	**04-08-25**	**0.9839**	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
10	**05-06-25**	**0.9680**	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
11	**06-03-25**	**1.0177**	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
12	**07-08-25**	**0.9124**	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
13	**08-05-25**	**0.9576**	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
14	**08-12-25**	**0.9003**	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
15	**09-09-25**	**0.9465**	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351
16	**10-07-25**	**0.9572**	-0.0190	-0.0200	0.0144	0.9549	0.8935	1.0205	0.8907	1.0236	0.7735	1.1363
17	**11-04-25**	**0.9840**	-0.0070	-0.0187	0.0143	0.9579	0.8967	1.0231	0.8940	1.0260	0.7759	1.1399
18	**12-02-25**	**0.9007**	-0.0454	-0.0204	0.0154	0.9542	0.8888	1.0243	0.8857	1.0277	0.7729	1.1355
19	**01-06-26**	**0.9716**	-0.0125	-0.0203	0.0154	0.9544	0.8888	1.0247	0.8857	1.0281	0.7730	1.1357
20	**02-10-26**	**1.0197**	0.0085	-0.0199	0.0161	0.9553	0.8869	1.0288	0.8838	1.0323	0.7738	1.1367

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

ETS

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 # 188

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 #: 2447

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	XL	XL	XL
Control, MHSW	pH (S.U.)	7.82	7.94	8.02
	Dissolved oxygen (mg/L)	8.4	8.1	8.2
	Conductivity (µmhos/cm)	295		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	25.4	24.9	25.1
500 mg/L	pH (S.U.)	8.00	7.91	8.00
	Dissolved oxygen (mg/L)	8.5	8.1	8.2
	Conductivity (µmhos/cm)	1150		
	Temperature (°C)	25.3	25.0	25.2
750 mg/L	pH (S.U.)	8.01	7.90	8.00
	Dissolved oxygen (mg/L)	8.6	8.1	8.2
	Conductivity (µmhos/cm)	1520		
	Temperature (°C)	25.0	25.0	25.2
1000 mg/L	pH (S.U.)	8.01	7.92	7.99
	Dissolved oxygen (mg/L)	8.6	8.1	8.2
	Conductivity (µmhos/cm)	1940		
	Temperature (°C)	25.4	25.1	24.9
1250 mg/L	pH (S.U.)	8.01	7.92	7.99
	Dissolved oxygen (mg/L)	8.6	8.1	8.2
	Conductivity (µmhos/cm)	2300		
	Temperature (°C)	25.5	25.1	25.2
1500 mg/L	pH (S.U.)	8.00	7.91	
	Dissolved oxygen (mg/L)	8.5	8.1	
	Conductivity (µmhos/cm)	2680		
	Temperature (°C)	25.1	25.0	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurement 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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	130647c

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 # 188

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	02-10-26	* 2540	JP	0805	JP	8 JP02-10-26 7C	Green	02-04-26B
24	02-11-26			0821	JP			
48 Termination	02-12-26			0813	JP			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	01-29-26
Age (1 to 14 days old):	5-6 DAYS
Hatch date and times:	02-04-26 1311 TO 02-05-26 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.26
	Temperature (°C): 25.1 C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	6 4d	6 4d	3 7d	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	10	9 1d	6	6	1 2d	1	0	0
Mean Survival	100 %		100 %		95%		60 %		10 %		0 %	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	945.5
Upper 95% confidence limit (mg KCl/L)	1089.7
48-hour LC_{50} (mg KCl/L)	1019.7

Comments:

Test Reviewed by: [signature]

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	2/10/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	2/12/2026	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	0.1000
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	0.2324	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2324	0	20
*1000	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	6.449	2.850	0.2324	8	20
*1250	0.2000	0.2000	0.4507	0.3218	0.5796	40.461	2	11.788	2.850	0.2324	16	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

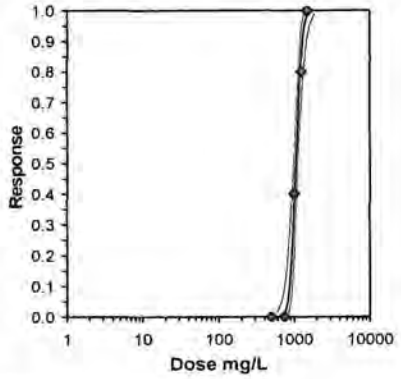
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.12039	0.12348	0.37918	0.00665	2.3E-04	4, 5
Treatments vs D-Control										

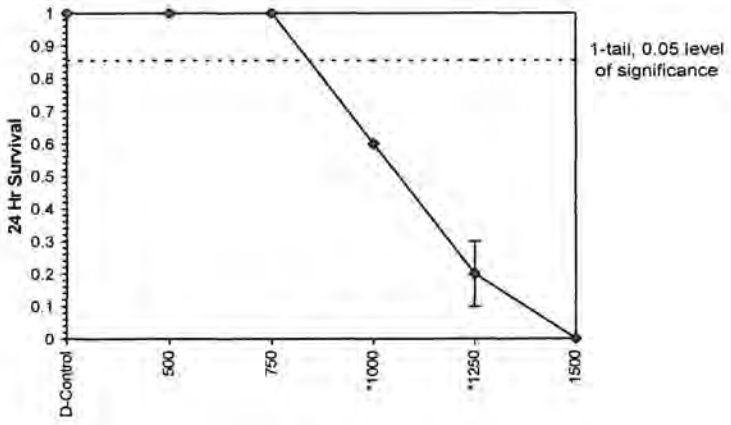
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.0382	2.88342	9.38669	20.6897	0	1.22962	7.81472	0.74591	3.02705	0.0665	5
Intercept	-40.521	8.76493	-57.701	-23.342							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	745.347	584.182	838.263
EC05	3.355	827.325	687.559	908.154
EC10	3.718	874.65	748.991	948.979
EC15	3.964	908.101	792.826	978.399
EC20	4.158	935.597	828.888	1003.15
EC25	4.326	959.848	860.54	1025.6
EC40	4.747	1023.78	941.81	1088.94
EC50	5.000	1064.28	990.22	1133.63
EC60	5.253	1106.37	1036.89	1184.97
EC75	5.674	1180.07	1109.23	1287.18
EC80	5.842	1210.65	1136.36	1333.6
EC85	6.036	1247.31	1167.24	1391.72
EC90	6.282	1295.01	1205.43	1470.73
EC95	6.645	1369.09	1261.62	1599.59
EC99	7.326	1519.67	1368.92	1879.75



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	2/10/2026	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	2/12/2026	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	0.9000
1000	0.6000	0.6000
1250	0.1000	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	0.1469	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	1.581	2.850	0.1469	1	20
*1000	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	2	10.205	2.850	0.1469	8	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	21.156	2.850	0.1469	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

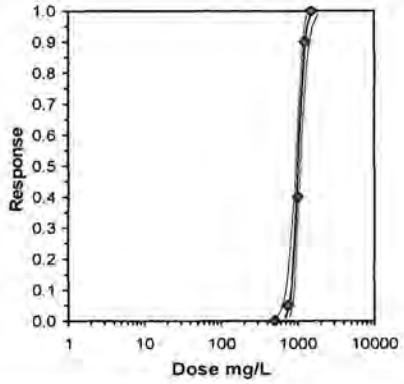
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.06555	0.06723	0.44775	0.00266	1.6E-05	4, 5
Treatments vs D-Control										

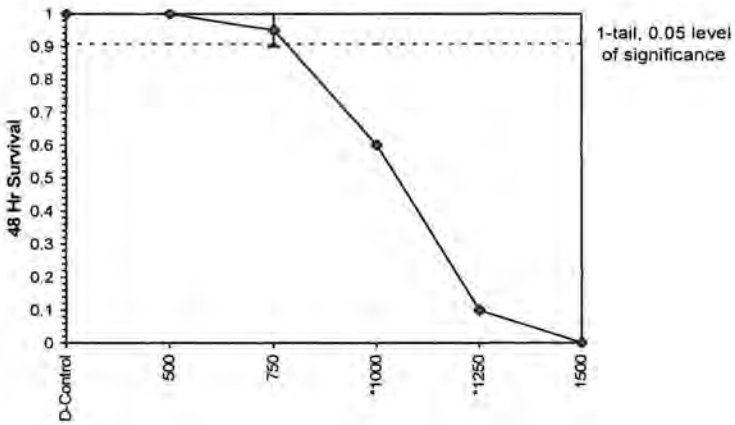
Maximum Likelihood-Probit

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

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

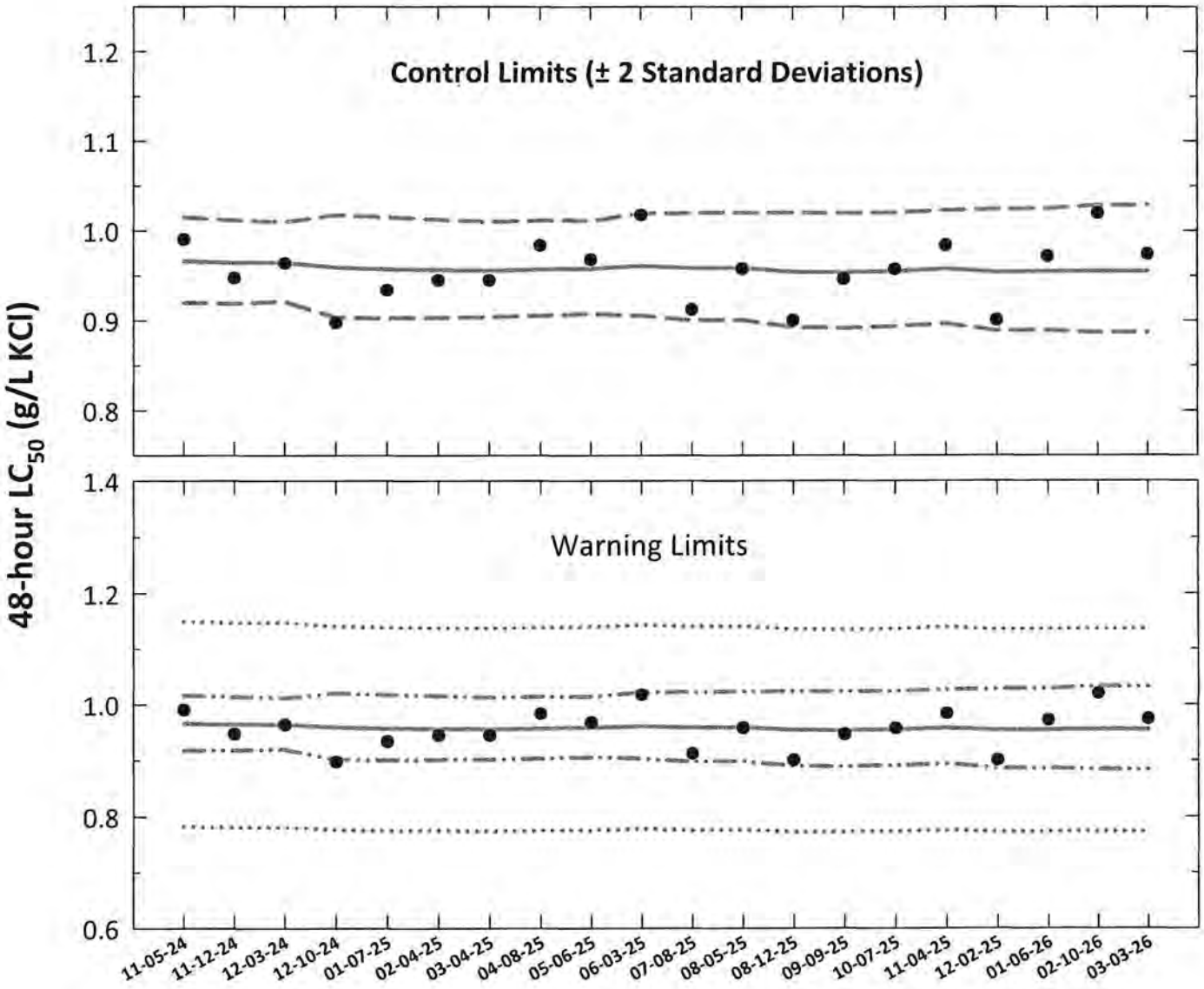


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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} ± $S_{A.75}$ 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	**11-05-24**	**0.9904**	-0.0042	-0.0149	0.0107	0.9662	0.9199	1.0148	0.9183	1.0165	0.7826	1.1498
2	**11-12-24**	**0.9473**	-0.0235	-0.0157	0.0104	0.9645	0.9192	1.0120	0.9175	1.0137	0.7812	1.1477
3	**12-03-24**	**0.9637**	-0.0160	-0.0157	0.0100	0.9644	0.9212	1.0097	0.9196	1.0113	0.7812	1.1476
4	**12-10-24**	**0.8977**	-0.0469	-0.0181	0.0129	0.9591	0.9039	1.0176	0.9016	1.0201	0.7769	1.1413
5	**01-07-25**	**0.9340**	-0.0296	-0.0190	0.0127	0.9573	0.9028	1.0151	0.9003	1.0177	0.7754	1.1392
6	**02-04-25**	**0.9448**	-0.0247	-0.0193	0.0124	0.9565	0.9035	1.0125	0.9011	1.0150	0.7747	1.1382
7	**03-04-25**	**0.9448**	-0.0247	-0.0197	0.0120	0.9557	0.9043	1.0101	0.9019	1.0126	0.7741	1.1373
8	**04-08-25**	**0.9839**	-0.0070	-0.0189	0.0120	0.9574	0.9058	1.0119	0.9035	1.0143	0.7755	1.1393
9	**05-06-25**	**0.9680**	-0.0141	-0.0187	0.0117	0.9579	0.9076	1.0111	0.9054	1.0134	0.7759	1.1400
10	**06-03-25**	**1.0177**	0.0076	-0.0173	0.0129	0.9610	0.9056	1.0198	0.9034	1.0222	0.7784	1.1436
11	**07-08-25**	**0.9124**	-0.0398	-0.0184	0.0135	0.9585	0.9006	1.0201	0.8981	1.0228	0.7764	1.1406
12	**08-05-25**	**0.9576**	-0.0188	-0.0184	0.0135	0.9585	0.9007	1.0201	0.8982	1.0228	0.7764	1.1406
13	**08-12-25**	**0.9003**	-0.0456	-0.0203	0.0145	0.9543	0.8925	1.0203	0.8896	1.0235	0.7730	1.1356
14	**09-09-25**	**0.9465**	-0.0239	-0.0205	0.0146	0.9538	0.8920	1.0199	0.8890	1.0231	0.7726	1.1351
15	**10-07-25**	**0.9572**	-0.0190	-0.0200	0.0144	0.9549	0.8935	1.0205	0.8907	1.0236	0.7735	1.1363
16	**11-04-25**	**0.9840**	-0.0070	-0.0187	0.0143	0.9579	0.8967	1.0231	0.8940	1.0260	0.7759	1.1399
17	**12-02-25**	**0.9007**	-0.0454	-0.0204	0.0154	0.9542	0.8888	1.0243	0.8857	1.0277	0.7729	1.1355
18	**01-06-26**	**0.9716**	-0.0125	-0.0203	0.0154	0.9544	0.8888	1.0247	0.8857	1.0281	0.7730	1.1357
19	**02-10-26**	**1.0197**	0.0085	-0.0199	0.0161	0.9553	0.8869	1.0288	0.8838	1.0323	0.7738	1.1367
20	**03-03-26**	**0.9741**	-0.0114	-0.0199	0.0161	0.9552	0.8870	1.0287	0.8838	1.0321	0.7737	1.1367

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

ETS

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 # 189

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 #: 2465

Chemical Analyses:

		Hours		
Concentration	Analyst	0	24	48
Control, MHSW	pH (S.U.)	8.16	7.95	7.89
	Dissolved oxygen (mg/L)	8.2	8.3	8.1
	Conductivity (µmhos/cm)	293		
	Alkalinity (mg/L $CaCO_3$)	63		
	Hardness (mg/L $CaCO_3$)	86		
	Temperature (°C)	24.2	25.4	25.2
500 mg/L	pH (S.U.)	8.04	7.93	7.86
	Dissolved oxygen (mg/L)	8.2	8.3	8.1
	Conductivity (µmhos/cm)	1110		
	Temperature (°C)	24.1	25.1	25.2
750 mg/L	pH (S.U.)	8.03	7.94	7.87
	Dissolved oxygen (mg/L)	8.2	8.3	8.1
	Conductivity (µmhos/cm)	1480		
	Temperature (°C)	24.6	25.2	24.9
1000 mg/L	pH (S.U.)	8.05	7.94	7.88
	Dissolved oxygen (mg/L)	8.3	8.3	8.1
	Conductivity (µmhos/cm)	1880		
	Temperature (°C)	24.3	25.2	24.9
1250 mg/L	pH (S.U.)	8.05	7.94	7.88
	Dissolved oxygen (mg/L)	8.3	8.2	8.0
	Conductivity (µmhos/cm)	2210		
	Temperature (°C)	24.6	24.9	24.7
1500 mg/L	pH (S.U.)	9.06	7.94	
	Dissolved oxygen (mg/L)	8.3	8.2	
	Conductivity (µmhos/cm)	2630		
	Temperature (°C)	24.4	25.4	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measuremer 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 th 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-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1306647

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	3/3/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/5/2026	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	0.7000	1.0000
1000	0.4000	0.8000
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	0.5571	0	20
750	0.8500	0.8500	1.2016	0.9912	1.4120	24.767	2	1.076	2.850	0.5571	3	20
1000	0.6000	0.6000	0.8959	0.6847	1.1071	33.340	2	2.640	2.850	0.5571	8	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	5.994	2.850	0.5571	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

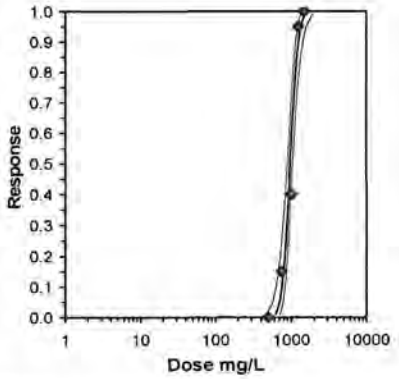
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.40573	0.41613	0.48147	0.03821	0.00801	4, 5
Treatments vs D-Control										

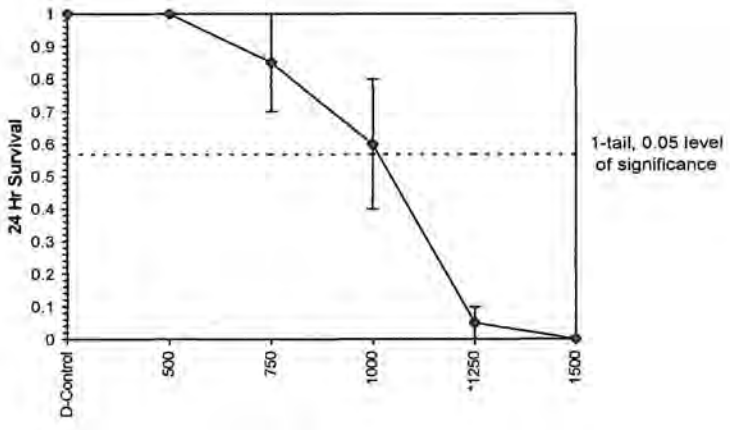
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.9118	2.11924	7.75807	16.0655	0	3.66713	7.81472	0.29972	2.98837	0.08395	3
Intercept	-30.597	6.37109	-43.084	-18.109							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	620.967	471.217	714.912
EC05	3.355	708.403	574.055	792.105
EC10	3.718	759.941	636.835	837.823
EC15	3.964	796.815	682.364	870.996
EC20	4.158	827.394	720.274	899.023
EC25	4.326	854.561	753.893	924.497
EC40	4.747	927.039	841.791	996.573
EC50	5.000	973.569	895.361	1047.47
EC60	5.253	1022.43	947.987	1106.02
EC75	5.674	1109.15	1031.62	1223.33
EC80	5.842	1145.57	1063.61	1277.11
EC85	6.036	1189.53	1100.39	1344.93
EC90	6.282	1247.25	1146.39	1438.02
EC95	6.645	1337.99	1215.04	1592.03
EC99	7.326	1526.39	1348.9	1935.64



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	3/3/2026	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/5/2026	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	0.9000
750	0.7000	1.0000
1000	0.4000	0.8000
1250	0.1000	0.0000
1500	0.0000	0.0000

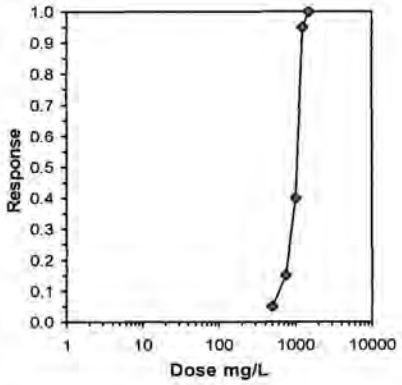
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.403	2.850	0.5762	1	20
750	0.8500	0.8500	1.2016	0.9912	1.4120	24.767	2	1.041	2.850	0.5762	3	20
1000	0.6000	0.6000	0.8959	0.6847	1.1071	33.340	2	2.553	2.850	0.5762	8	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	5.796	2.850	0.5762	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				

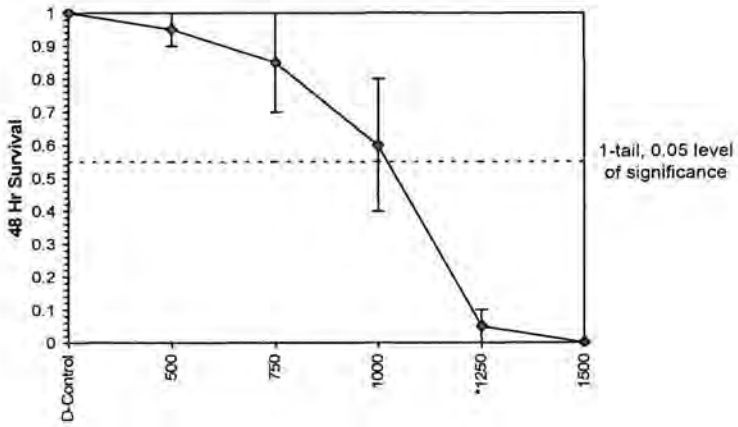
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1250	1118.03		0.42462	0.43551	0.45319	0.04087	0.01061	4, 5
Treatments vs D-Control										

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	974.078	889.379	1066.84
10.0%	994.144	906.537	1090.22
20.0%	1015.86	922.866	1118.23
Auto-5.0%	974.078	889.379	1066.84

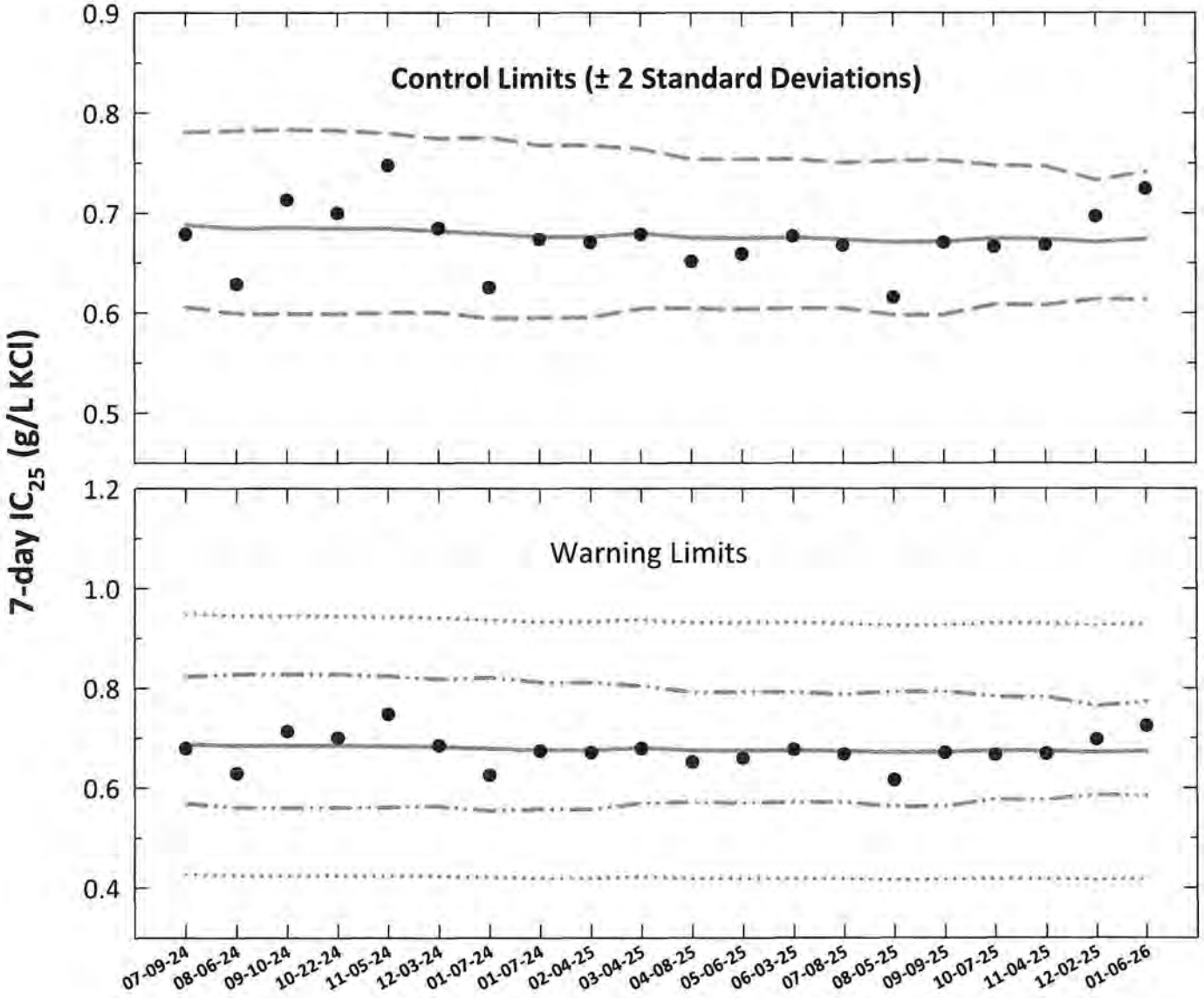


Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

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 $IC_{25} \pm S_{A.75}$ 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	07-09-24	0.6789	-0.1682	-0.1624	0.0276	0.6880	0.6060	0.7811	0.5688	0.8233	0.4265	0.9494
2	08-06-24	0.6287	-0.2015	-0.1644	0.0289	0.6848	0.5994	0.7823	0.5602	0.8272	0.4246	0.9450
3	09-10-24	0.7128	-0.1470	-0.1642	0.0290	0.6852	0.5994	0.7832	0.5600	0.8283	0.4248	0.9456
4	10-22-24	0.6997	-0.1551	-0.1645	0.0289	0.6847	0.5994	0.7822	0.5601	0.8271	0.4245	0.9449
5	11-05-24	0.7473	-0.1265	-0.1648	0.0284	0.6842	0.6004	0.7797	0.5617	0.8238	0.4242	0.9442
6	12-03-24	0.6844	-0.1647	-0.1662	0.0276	0.6820	0.6006	0.7745	0.5626	0.8176	0.4228	0.9411
7	01-07-24	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946	0.7757	0.5547	0.8213	0.4211	0.9372
8	01-07-24	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955	0.7675	0.5569	0.8113	0.4191	0.9329
9	02-04-25	0.6706	-0.1735	-0.1699	0.0275	0.6762	0.5956	0.7676	0.5571	0.8114	0.4192	0.9331
10	03-04-25	0.6786	-0.1684	-0.1676	0.0254	0.6798	0.6047	0.7643	0.5693	0.8041	0.4215	0.9381
11	04-08-25	0.6516	-0.1860	-0.1705	0.0239	0.6753	0.6048	0.7540	0.5708	0.7919	0.4187	0.9319
12	05-06-25	0.6589	-0.1812	-0.1709	0.0241	0.6746	0.6039	0.7537	0.5697	0.7918	0.4183	0.9310
13	06-03-25	0.6768	-0.1695	-0.1703	0.0239	0.6757	0.6053	0.7543	0.5715	0.7920	0.4189	0.9324
14	07-08-25	0.6674	-0.1756	-0.1716	0.0234	0.6736	0.6048	0.7501	0.5715	0.7872	0.4176	0.9295
15	08-05-25	0.6156	-0.2107	-0.1734	0.0249	0.6709	0.5981	0.7525	0.5623	0.7926	0.4159	0.9258
16	09-09-25	0.6703	-0.1738	-0.1731	0.0249	0.6712	0.5985	0.7529	0.5628	0.7928	0.4162	0.9263
17	10-07-25	0.6661	-0.1765	-0.1709	0.0223	0.6747	0.6087	0.7477	0.5769	0.7829	0.4183	0.9310
18	11-04-25	0.6681	-0.1752	-0.1715	0.0223	0.6738	0.6081	0.7466	0.5763	0.7819	0.4177	0.9298
19	12-02-25	0.6967	-0.1570	-0.1733	0.0192	0.6710	0.6141	0.7331	0.5862	0.7636	0.4160	0.9260
20	01-06-26	0.7242	-0.1401	-0.1713	0.0205	0.6741	0.6133	0.7409	0.5839	0.7732	0.4179	0.9302

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.

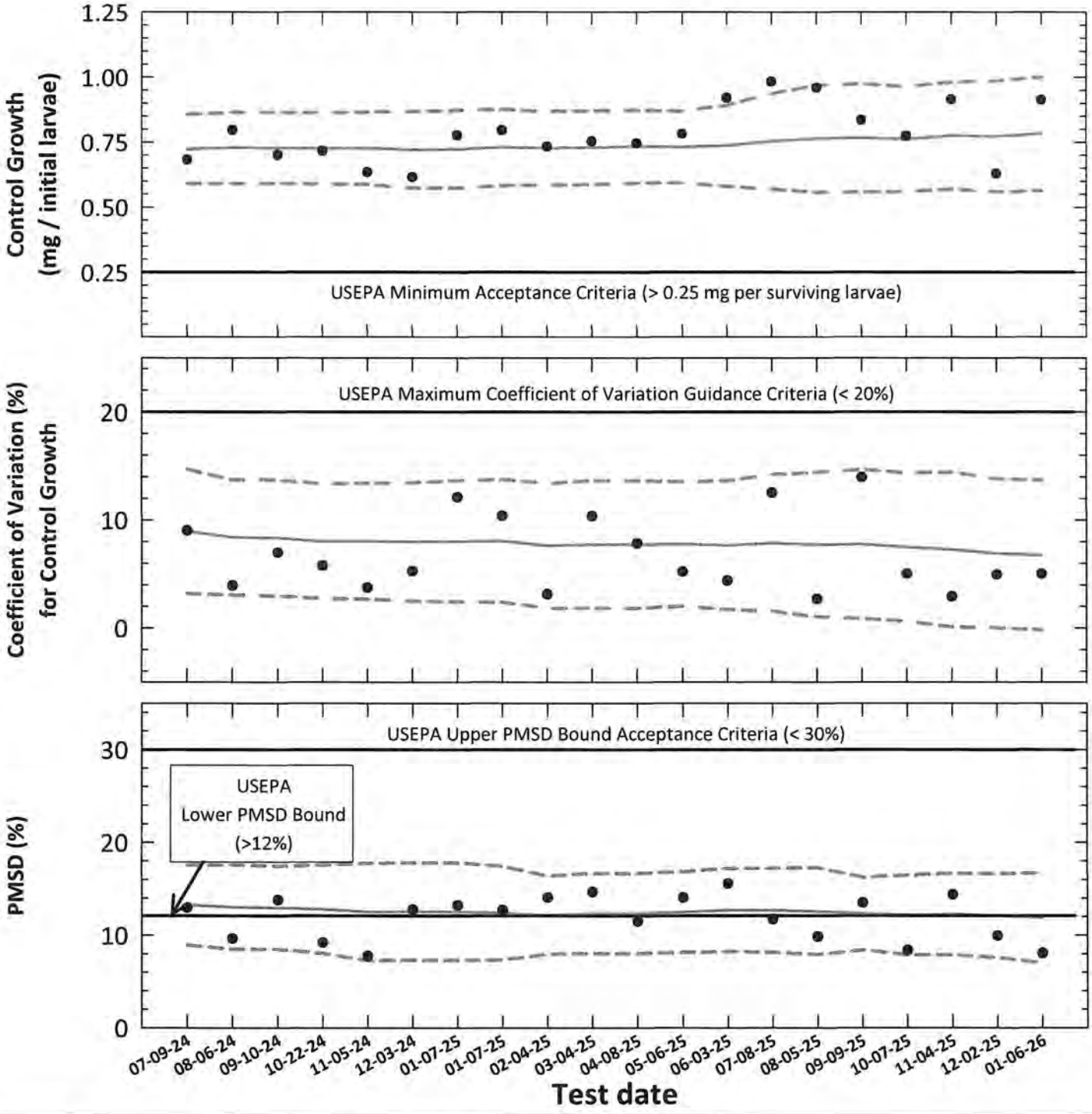
Entered and Reviewed by Jim Sumner

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		CT	95% Confidence Interval		CT	95% Confidence Interval		CT	95% Confidence Interval	
			Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)		CT - 2S	CT + 2S		CT - 2S	CT + 2S		CT - 2S	CT + 2S
1	07-09-24	100	0.683	9.0	0.0883	12.9	0.724	0.591	0.857	9.0	3.2	14.7	13.2	8.9	17.5
2	08-06-24	100	0.796	3.9	0.0766	9.6	0.728	0.591	0.865	8.4	3.0	13.7	13.0	8.4	17.5
3	09-10-24	100	0.701	7.0	0.0964	13.8	0.728	0.591	0.865	8.3	2.9	13.7	12.9	8.5	17.4
4	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.8	8.0	17.6
5	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.5	7.3	17.8
6	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
7	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
8	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	12.4	7.3	17.4
9	02-04-25	100	0.733	3.1	0.1030	14.1	0.727	0.585	0.869	7.6	1.8	13.4	12.2	7.9	16.4
10	03-04-25	100	0.754	10.4	0.1103	14.6	0.729	0.588	0.871	7.7	1.8	13.6	12.3	8.0	16.7
11	04-08-25	100	0.746	7.8	0.0855	11.5	0.733	0.593	0.873	7.7	1.8	13.7	12.3	8.0	16.7
12	05-06-25	100	0.783	5.2	0.1099	14.0	0.732	0.593	0.871	7.8	2.0	13.6	12.5	8.1	16.8
13	06-03-25	100	0.920	4.4	0.1429	15.5	0.736	0.580	0.893	7.7	1.7	13.6	12.7	8.2	17.2
14	07-08-25	100	0.983	12.5	0.1150	11.7	0.754	0.570	0.938	7.9	1.5	14.2	12.7	8.1	17.2
15	08-05-25	100	0.959	2.7	0.0941	9.8	0.763	0.558	0.968	7.7	1.0	14.4	12.6	7.9	17.2
16	09-09-25	100	0.835	14.0	0.1126	13.5	0.767	0.559	0.975	7.8	0.9	14.7	12.3	8.4	16.2
17	10-07-25	100	0.774	5.0	0.0646	8.3	0.762	0.560	0.964	7.5	0.6	14.4	12.2	7.9	16.5
18	11-04-25	100	0.914	2.9	0.1313	14.4	0.775	0.569	0.981	7.3	0.1	14.4	12.2	7.8	16.6
19	12-02-25	100	0.629	5.0	0.0623	9.9	0.772	0.559	0.985	6.9	0.0	13.8	12.1	7.6	16.6
20	01-06-26	100	0.914	5.0	0.0736	8.1	0.783	0.564	1.002	6.8	-0.2	13.7	11.9	7.0	16.7

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 the Supervisor

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKCICR Test Number: **136**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2447				
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:	12-30-25 1521	*Artemia* CHM number:	CHM1385
Hatch dates and times:	01-05-26 A TO 01-06-26 0600	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.21 S.U. Temperature = 24.0 °C	Date / Time in oven:	01-13-26 0645
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	01-14-26 0645
		*Final oven temperature:	60°C
		Total drying time:	24-HOURS

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	01-06-26	0605	JK	1340	JK	0605	JK	12-30-25A
1	01-07-26	0500	JK	1100	JK	0700	JK	↓
2	01-08-26	0500	JK	1100	JK	0700	JK	↓
3	01-09-26	0500	JK	1100	JK	0700	JK	12-30-25 B
4	01-10-26	0700	JK	1230	JK	0900	JK	↓
5	01-11-26	0600	JK	1130	JK	0800	JK	01-08-25 A
6	01-12-26	0600	JK	1200	JK	0800	JK	↓
7	01-13-26					0645	JK	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664665

Control information:		Acceptance criteria
% Mortality:	0%	≤ 20%
Average weight per initial larvae:	0.914	
Average weight per surviving larvae:	0.914	≥ 0.25 mg/larvae

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	628.9
NOEC (mg/L KCl)	600
LOEC (mg/L KCl)	750
ChV (mg/L KCl)	670.8
IC_{25} (mg/L KCl)	724.2

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **136**

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: Light pink Analyst: XL Date: 12-17-25	14.40	13.44	14.01	10.85	14.27	12.98	13.31	12.28	13.38	13.81	10.10	12.50
*B = Pan + Larvae weight (mg) Analyst: JL Date: 01-15-26	23.58	21.96	23.23	20.48	25.07	23.45	23.84	22.58	21.65	22.27	18.80	21.48
C = Larvae weight (mg) = B – A Analyst: JL	9.18	8.52	9.22	9.63	10.80	10.47	10.53	10.30	8.27	8.46	8.70	8.98
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.918	0.852	0.922	0.963	1.080	1.047	1.053	1.030	0.827	0.846	0.870	0.898
Average weight per initial number of larvae (mg)	0.914				1.053				0.860			
Percent reduction from control (%)					-15.22				5.97			

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **136**

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	10	10	7 3d	8 2d	7 3d	7 3d
2	10	10	10	10	9 1d	9	9 1d	9 1d	6 1d	6 2d	5 2d	6 1d
3	10	10	10	10	9	9	8 1d	9	6	6	5	6
4	10	10	10	10	7 2d	9	8	9	4 2d	4 2d	4 2d	4 2d
5	10	10	10	10	7	8 1d	8	7 2d	4	4	4	4
6	10	10	10	10	7	8	8	7	3 1d	4	4	3 1d
7	9 1d	10	10	9 1d	7	8	8	7	3	3 1d	4	3
*A = Pan weight (mg) Tray color code: Light pink Analyst: XL Date: 12-17-25	12.45	15.10	12.23	13.85	11.89	13.96	13.47	13.44	10.67	12.93	12.79	12.61
*B = Pan + Larvae weight (mg) Analyst: JL Date: 01-15-26	21.08	25.32	~~25.32~~ 22.00	~~22.00~~ 23.04	~~22.01~~ 18.15	~~25.04~~ 20.75	~~18.15~~ 21.47	~~20.38~~ 20.51	14.03	16.68	17.70	15.77
C = Larvae weight (mg) = B – A Analyst: JL	8.63	10.22	9.77 CONFIRMED	9.19	6.26 JL.	6.79	8.00	7.07	3.36	3.75	4.91	3.16
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.863	1.022	0.977	0.919	0.626	0.679	0.800	0.707	0.336	0.375	0.491	0.316
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.945		-3.4%		0.703		23.1%		0.380		58.5%	

*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:

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **136**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	2 8d	3 7d	2 8d	3 7d
2	2	2 1d	2	1 2d
3	2	2	2	1
4	2	2	1 1d	1
5	1 1d	1 1d	0 1d	1
6	1	1	0	1
7	1	1	0	1
*A = Pan weight (mg) Tray color code: light pink Analyst: XL Date: 12-17-25	11.83	13.47	13.34	13.80
*B = Pan + Larvae weight (mg) Analyst: JK Date: 01-15-26	01-15-26 ~~+3.77~~ 13.06	15.05	—	15.05
C = Larvae weight (mg) = B – A Analyst: JK	1.23	1.58	JK 01-15-26	1.25
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JK	0.123	0.158	0	0.125
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.102		88.9 %	

*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.

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: 136

Test dates: January 06-13, 2026

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	14.40	23.58	9.18	0.918	0.914	5.0	0.918	100.0	0.914	5.0	Not applicable
	B	10	10	13.44	21.96	8.52	0.852			0.852				
	C	10	10	14.01	23.23	9.22	0.922			0.922				
	D	10	10	10.85	20.48	9.63	0.963			0.963				
300	E	10	10	14.27	25.07	10.80	1.080	1.053	2.0	1.080	100.0	1.053	2.0	-15.2
	F	10	10	12.98	23.45	10.47	1.047			1.047				
	G	10	10	13.31	23.84	10.53	1.053			1.053				
	H	10	10	12.28	22.58	10.30	1.030			1.030				
450	I	10	10	13.38	21.65	8.27	0.827	0.860	3.6	0.827	100.0	0.860	3.6	5.9
	J	10	10	13.81	22.27	8.46	0.846			0.846				
	K	10	10	10.10	18.80	8.70	0.870			0.870				
	L	10	10	12.50	21.48	8.98	0.898			0.898				
600	M	10	9	12.45	21.08	8.63	0.959	0.995	3.2	0.863	95.0	0.945	7.3	-3.4
	N	10	10	15.10	25.32	10.22	1.022			1.022				
	O	10	10	12.23	22.00	9.77	0.977			0.977				
	P	10	9	13.85	23.04	9.19	1.021			0.919				
750	Q	10	7	11.89	18.15	6.26	0.894	0.938	8.5	0.626	75.0	0.703	10.4	23.1
	R	10	8	13.96	20.75	6.79	0.849			0.679				
	S	10	8	13.47	21.47	8.00	1.000			0.800				
	T	10	7	13.44	20.51	7.07	1.010			0.707				
900	U	10	3	10.67	14.03	3.36	1.120	1.163	7.9	0.336	32.5	0.380	20.6	58.5
	V	10	3	12.93	16.68	3.75	1.250			0.375				
	W	10	4	12.79	17.70	4.91	1.228			0.491				
	X	10	3	12.61	15.77	3.16	1.053			0.316				
1050	Y	10	1	11.83	13.06	1.23	1.230	1.353	14.5	0.123	7.5	0.102	68.5	88.9
	Z	10	1	13.47	15.05	1.58	1.580			0.158				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
	BB	10	1	13.80	15.05	1.25	1.250			0.125				

Dunnett's MSD value: 0.0736 MSD = Minimum Significant Difference

PMSD: 8.1 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.

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	1/6/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	1/13/2026	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	0.9000
750	0.7000	0.8000	0.8000	0.7000
900	0.3000	0.3000	0.4000	0.3000
1050	0.1000	0.1000	0.0000	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	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.7500	0.7500	1.0492	0.9912	1.1071	6.383	4	10.00	10.00	10	40
*900	0.3250	0.3250	0.6059	0.5796	0.6847	8.671	4	10.00	10.00	27	40
*1050	0.0750	0.0750	0.2810	0.1588	0.3218	28.997	4	10.00	10.00	37	40

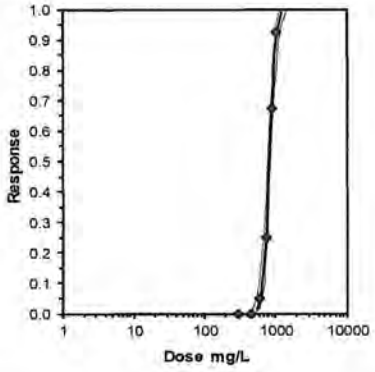
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92623	0.896	-0.3732	0.20944
Equality of variance cannot be confirmed				

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

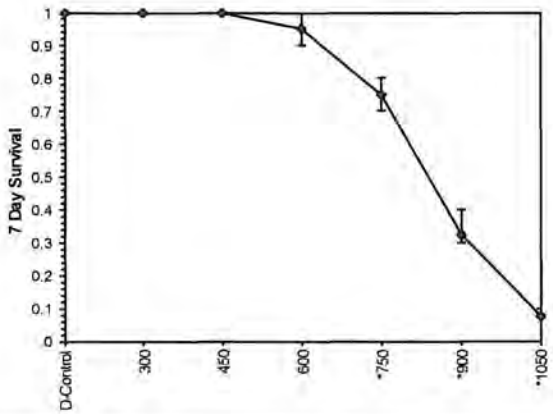
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.0841	1.68548	9.78056	16.3876	0	0.71175	9.48773	0.94987	2.91848	0.07643	3
Intercept	-33.186	4.92523	-42.839	-23.532							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	550.405	474.347	603.505
EC05	3.355	620.537	555.232	666.141
EC10	3.718	661.507	603.246	702.847
EC15	3.964	690.667	637.521	729.256
EC20	4.158	714.756	665.743	751.404
EC25	4.326	736.091	690.543	771.394
EC40	4.747	792.718	754.457	827.163
EC50	5.000	828.861	792.89	865.716
EC60	5.253	866.652	830.596	908.993
EC75	5.674	933.323	891.637	992.02
EC80	5.842	961.182	915.621	1028.68
EC85	6.036	994.707	943.665	1073.95
EC90	6.282	1038.55	979.341	1134.71
EC95	6.645	1107.12	1033.52	1232.58
EC99	7.326	1248.19	1141.01	1442.48



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and formatted by Jim Lechner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	1/6/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxcant
End Date:	1/13/2026	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	0.9180	0.8520	0.9220	0.9630
300	1.0800	1.0470	1.0530	1.0300
450	0.8270	0.8460	0.8700	0.8980
600	0.8630	1.0220	0.9770	0.9190
750	0.6260	0.6790	0.8000	0.7070
900	0.3360	0.3750	0.4910	0.3160
1050	0.1230	0.1580	0.0000	0.1250

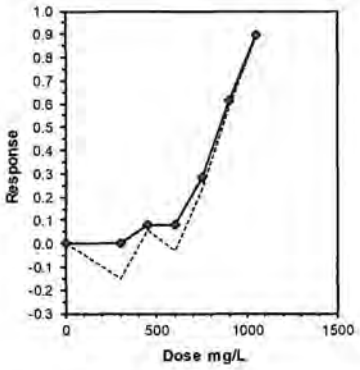
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.9138	1.0000	0.9138	0.8520	0.9630	5.025	4				0.9831	1.0000
300	1.0525	1.1518	1.0525	1.0300	1.0800	1.972	4	-4.316	2.290	0.0736	0.9831	1.0000
450	0.8603	0.9415	0.8603	0.8270	0.8980	3.570	4	1.664	2.290	0.0736	0.9028	0.9182
600	0.9453	1.0345	0.9453	0.8630	1.0220	7.317	4	-0.980	2.290	0.0736	0.9028	0.9182
750	0.7030	0.7694	0.7030	0.6260	0.8000	10.366	4				0.7030	0.7151
900	0.3795	0.4153	0.3795	0.3160	0.4910	20.824	4				0.3795	0.3860
1050	0.1015	0.1111	0.1015	0.0000	0.1580	68.516	4				0.1015	0.1032

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98876	0.844	-0.2057	0.17384
Bartlett's Test indicates equal variances (p = 0.27)	3.93242	11.3449		

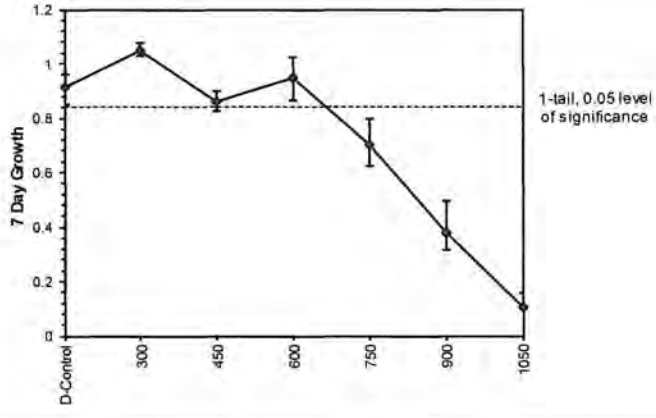
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.07361	0.08056	0.02626	0.00207	4.9E-04	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	391.74	45.65	348.49	727.94	3.4422
IC10	613.47	64.68	318.99	653.26	-1.9503
IC15	650.38	14.12	613.55	692.26	0.1962
IC20	687.30	17.85	643.62	756.25	0.6795
IC25	724.21	19.50	670.83	785.31	0.0846
IC40	802.45	12.29	763.92	841.77	0.1525
IC50	848.04	13.01	815.34	897.04	0.9118



Dose-Response Plot



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

Test type:	***P. promelas* Chronic Reference Toxicant Test**
Test dates:	**January 06-13, 2026**
Associated test:	**PpKClCR # 136**

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

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

	1st Weight	2nd Weight	Difference (mg)	Percent Difference from 1st Weight (%)
Analyst:		JP		
Tray color code:		Light Pink		
Date:		01-16-26		
A		23.59		
B		21.97		
C		23.24		
D		20.52		
E		25.08		
F		23.47		
G		23.86		
H		22.62		
I		21.67		
J		22.30		
K		18.82		
L		21.52		
M		21.11		
N		25.34		
O		22.02		
P		23.06		
Q		~~18.14~~ 18.15 JP 01-16-26		
R		20.74		
S		21.51		
T		20.54		
U		14.05		
V		16.68		
W		17.71		
X		15.77		
Y		13.06		
Z		15.04		
AA		13.35		
BB		15.05		
		Average		

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

Test type:	***P. promelas* Chronic Reference Toxicant Test**
Test dates:	**January 06-13, 2026**
Associated test:	**PpKClCR # 124**

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

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

	1st Weight	2nd Weight		
Analyst:	JS	JP	Difference (mg)	Percent Difference from 1st Weight (%)
Tray color code:	Light Pink	Light Pink		
Date:	01-15-26	01-16-26		
A	23.58	23.59	0.01	0.04
B	21.96	21.97	0.01	0.05
C	23.23	23.24	0.01	0.04
D	20.48	20.52	0.04	0.20
E	25.07	25.08	0.01	0.04
F	23.45	23.47	0.02	0.09
G	23.84	23.86	0.02	0.09
H	22.58	22.62	0.04	0.18
I	21.65	21.67	0.02	0.09
J	22.27	22.30	0.03	0.13
K	18.80	18.82	0.02	0.11
L	21.48	21.52	0.04	0.19
M	21.08	21.11	0.03	0.14
N	25.32	25.34	0.02	0.08
O	22.00	22.02	0.02	0.09
P	23.04	23.06	0.02	0.09
Q	18.15	18.15	0.00	0.00
R	20.75	20.74	-0.01	-0.05
S	21.47	21.51	0.04	0.19
T	20.51	20.54	0.03	0.15
U	14.03	14.05	0.02	0.14
V	16.68	16.68	0.00	0.00
W	17.70	17.71	0.01	0.06
X	15.77	15.77	0.00	0.00
Y	13.06	13.06	0.00	0.00
Z	15.05	15.04	-0.01	-0.07
AA	13.34	13.35	0.01	0.07
BB	15.05	15.05	0.00	0.00
		Average	0.02	0.08

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **136**

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	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.84	7.82	7.73	7.71	7.79	7.76
	Dissolved oxygen (mg/L)	8.5	7.8	8.2	7.9	8.4	7.9
	Conductivity (µmhos/cm)	289		305		300	
	Alkalinity (mg $CaCO_3$/L)	63					
	Hardness (mg $CaCO_3$/L)	90					
	Temperature (°C)	24.8	25.3	25.0	25.1	24.8	25.1
300 mg KCl/L	pH (S.U.)	7.94	7.83	7.95	7.71	7.91	7.73
	Dissolved oxygen (mg/L)	8.4	7.9	8.2	7.9	8.3	7.8
	Conductivity (µmhos/cm)	785		881		820	
	Temperature (°C)	24.7	25.1	25.1	24.8	24.7	24.9
450 mg KCl/L	pH (S.U.)	7.96	7.84	7.95	7.72	7.92	7.73
	Dissolved oxygen (mg/L)	8.5	7.9	8.2	7.8	8.3	7.8
	Conductivity (µmhos/cm)	1010		1070		1050	
	Temperature (°C)	24.7	25.0	25.1	24.8	24.7	24.9
600 mg KCl/L	pH (S.U.)	7.97	7.84	7.96	7.73	7.93	7.73
	Dissolved oxygen (mg/L)	8.5	7.9	8.3	7.8	8.3	7.8
	Conductivity (µmhos/cm)	1250		1320		1300	
	Temperature (°C)	24.9	25.0	24.9	25.2	24.8	25.0
750 mg KCl/L	pH (S.U.)	7.98	7.84	7.96	7.73	7.93	7.74
	Dissolved oxygen (mg/L)	8.5	7.8	8.3	7.8	8.4	7.7
	Conductivity (µmhos/cm)	1500		1550		1540	
	Temperature (°C)	24.9	24.9	24.9	25.2	24.6	25.0
900 mg KCl/L	pH (S.U.)	7.98	7.83	7.96	7.74	7.93	7.76
	Dissolved oxygen (mg/L)	8.4	7.8	8.3	7.8	8.4	7.8
	Conductivity (µmhos/cm)	1710		1800		1770	
	Temperature (°C)	24.9	24.9	24.8	25.0	24.6	24.8
1050 mg KCl/L	pH (S.U.)	7.98	7.83	7.97	7.76	7.94	7.78
	Dissolved oxygen (mg/L)	8.4	7.7	8.3	7.9	8.4	7.9
	Conductivity (µmhos/cm)	1970		2040		2030	
	Temperature (°C)	24.8	24.9	25.0	24.9	24.9	24.8
		Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

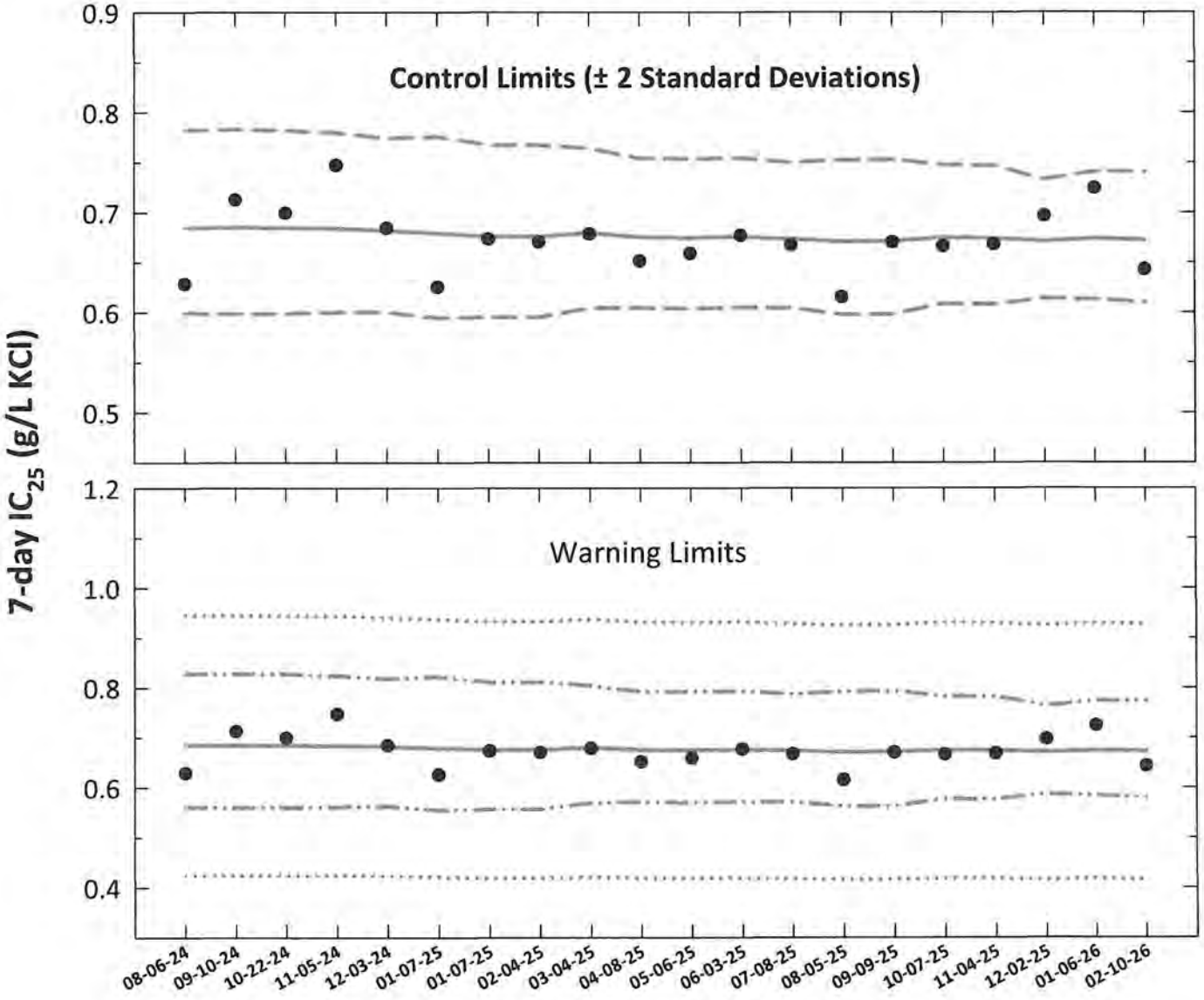
Species: *Pimephales promelas* PpKClCR Test Number: **136**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	**Parameter**								
CONTROL, MHSW	pH (S.U.)	7.75	7.73	7.67	8.0 (7.46)	7.84	7.61	7.89	7.60
	Dissolved oxygen (mg/L)	8.3	7.6	8.5	*8.0 (7.3)	8.3	7.3	8.4	6.1
	Conductivity (µmhos/cm)	~~30~~ 298		280		298		301	
	Alkalinity (mg $CaCO_3$/L)	63				62			
	Hardness (mg $CaCO_3$/L)	90				86			
	Temperature (°C)	24.8	25.2	24.7	25.0	24.7	25.2	24.7	24.9
300 mg KCl/L	pH (S.U.)	7.86	7.69	7.91	7.63	8.02	7.61	7.99	7.63
	Dissolved oxygen (mg/L)	8.2	7.5	8.3	*8.0 (6.8)	8.2	7.3	8.4	5.4
	Conductivity (µmhos/cm)	810		772		783		799	
	Temperature (°C)	24.9	24.9	24.9	24.9	24.8	25.0	24.8	24.4
450 mg KCl/L	pH (S.U.)	7.86	7.69	7.93	7.64	8.03	7.62	8.01	7.61
	Dissolved oxygen (mg/L)	8.2	7.5	8.3	*8.0 (6.9)	8.2	7.2	8.4	5.2
	Conductivity (µmhos/cm)	1040		1000		1010		1020	
	Temperature (°C)	24.8	25.0	24.9	24.9	24.8	25.0	24.8	24.9
600 mg KCl/L	pH (S.U.)	7.87	7.69	7.93	7.65	8.03	7.63	8.01	7.58
	Dissolved oxygen (mg/L)	8.2	7.5	8.3	*8.0 (7.0)	8.2	7.3	8.4	5.3
	Conductivity (µmhos/cm)	1290		1230		1260		1260	
	Temperature (°C)	24.8	25.0	24.8	25.0	25.0	25.0	24.9	24.7
750 mg KCl/L	pH (S.U.)	7.87	7.69	7.93	7.64	8.04	7.64	8.02	7.58
	Dissolved oxygen (mg/L)	8.2	7.5	8.3	*7.9 (6.9)	8.2	7.4	8.4	5.4
	Conductivity (µmhos/cm)	1510		1450		1500		1510	
	Temperature (°C)	24.9	25.2	24.8	24.8	24.8	24.9	24.8	24.7
900 mg KCl/L	pH (S.U.)	7.88	7.76	7.93	7.64	8.04	7.65	8.02	7.56
	Dissolved oxygen (mg/L)	8.2	7.7	8.3	*7.9 (7.0)	8.2	7.5	8.4	5.4
	Conductivity (µmhos/cm)	1760		1690		1750		1760	
	Temperature (°C)	24.9	24.9	24.8	25.1	24.8	24.9	24.8	24.6
1050 mg KCl/L	pH (S.U.)	7.88	7.76	7.93	7.70	8.04	7.67	8.02	7.55
	Dissolved oxygen (mg/L)	8.2	7.7	8.3	*7.9 (7.1)	8.1	7.4	8.4	5.9
	Conductivity (µmhos/cm)	2020		1950		1970		1990	
	Temperature (°C)	24.9	24.9	25.0	24.9	24.9	24.7	24.8	24.6
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

* BSL 01-11-26 verified correct

ETS
Environmental Testing Solutions, Inc.

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 $IC_{25} \pm S_{A.75}$ 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	08-06-24	0.6287	-0.2015	-0.1644	0.0289	0.6848	0.5994	0.7823	0.5602	0.8272	0.4246	0.9450
2	09-10-24	0.7128	-0.1470	-0.1642	0.0290	0.6852	0.5994	0.7832	0.5600	0.8283	0.4248	0.9456
3	10-22-24	0.6997	-0.1551	-0.1645	0.0289	0.6847	0.5994	0.7822	0.5601	0.8271	0.4245	0.9449
4	11-05-24	0.7473	-0.1265	-0.1648	0.0284	0.6842	0.6004	0.7797	0.5617	0.8238	0.4242	0.9442
5	12-03-24	0.6844	-0.1647	-0.1662	0.0276	0.6820	0.6006	0.7745	0.5626	0.8176	0.4228	0.9411
6	01-07-25	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946	0.7757	0.5547	0.8213	0.4211	0.9372
7	01-07-25	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955	0.7675	0.5569	0.8113	0.4191	0.9329
8	02-04-25	0.6706	-0.1735	-0.1699	0.0275	0.6762	0.5956	0.7676	0.5571	0.8114	0.4192	0.9331
9	03-04-25	0.6786	-0.1684	-0.1676	0.0254	0.6798	0.6047	0.7643	0.5693	0.8041	0.4215	0.9381
10	04-08-25	0.6516	-0.1860	-0.1705	0.0239	0.6753	0.6048	0.7540	0.5708	0.7919	0.4187	0.9319
11	05-06-25	0.6589	-0.1812	-0.1709	0.0241	0.6746	0.6039	0.7537	0.5697	0.7918	0.4183	0.9310
12	06-03-25	0.6768	-0.1695	-0.1703	0.0239	0.6757	0.6053	0.7543	0.5715	0.7920	0.4189	0.9324
13	07-08-25	0.6674	-0.1756	-0.1716	0.0234	0.6736	0.6048	0.7501	0.5715	0.7872	0.4176	0.9295
14	08-05-25	0.6156	-0.2107	-0.1734	0.0249	0.6709	0.5981	0.7525	0.5623	0.7926	0.4159	0.9258
15	09-09-25	0.6703	-0.1738	-0.1731	0.0249	0.6712	0.5985	0.7529	0.5628	0.7928	0.4162	0.9263
16	10-07-25	0.6661	-0.1765	-0.1709	0.0223	0.6747	0.6087	0.7477	0.5769	0.7829	0.4183	0.9310
17	11-04-25	0.6681	-0.1752	-0.1715	0.0223	0.6738	0.6081	0.7466	0.5763	0.7819	0.4177	0.9298
18	12-02-25	0.6967	-0.1570	-0.1733	0.0192	0.6710	0.6141	0.7331	0.5862	0.7636	0.4160	0.9260
19	01-06-26	0.7242	-0.1401	-0.1713	0.0205	0.6741	0.6133	0.7409	0.5839	0.7732	0.4179	0.9302
20	02-10-26	0.6431	-0.1917	-0.1725	0.0210	0.6722	0.6103	0.7405	0.5800	0.7738	0.4168	0.9277

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.

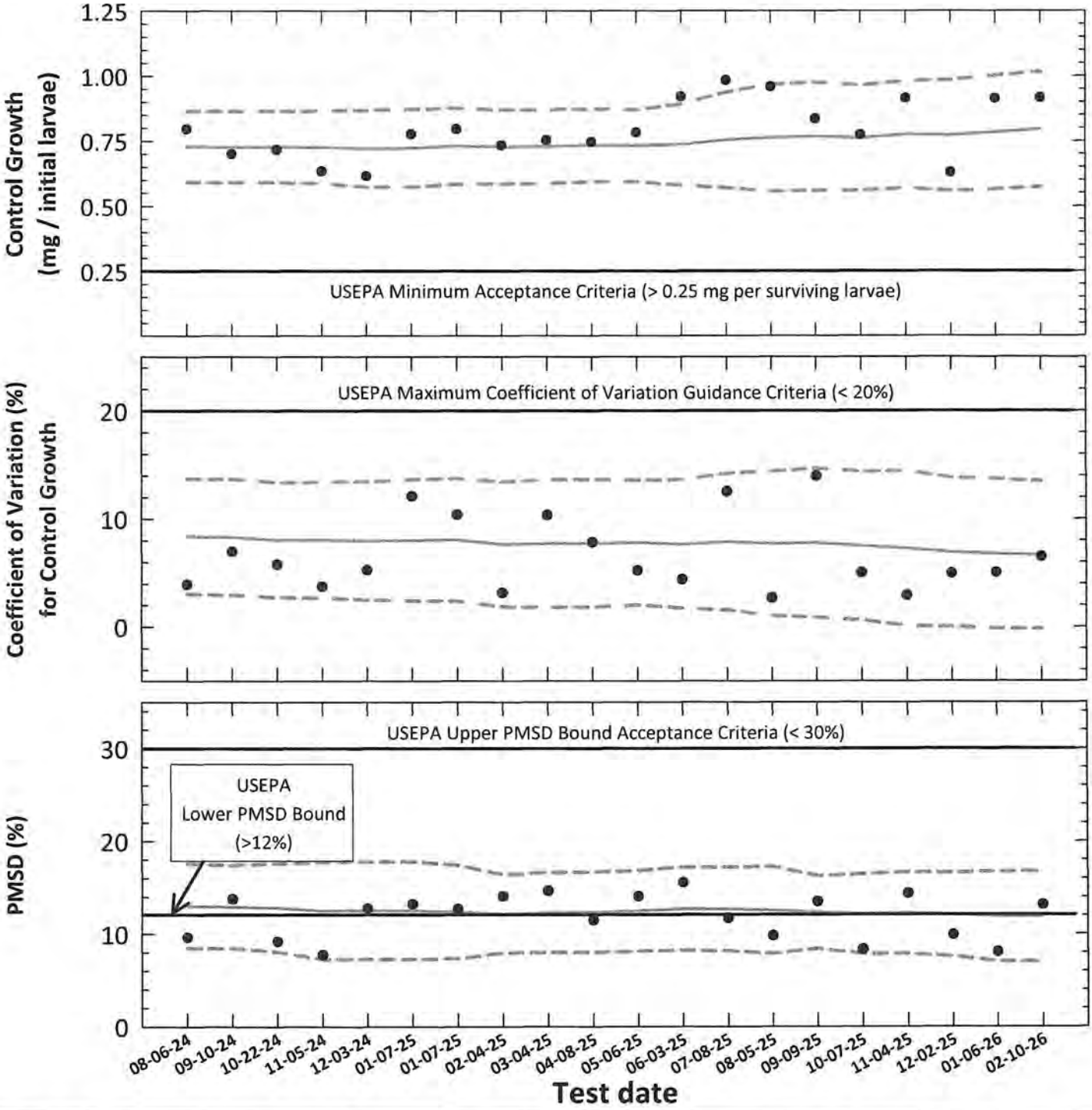
Entered and Reviewed by Jim Sumner

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

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			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	08-06-24	100	0.796	3.9	0.0766	9.6	0.728	0.591	0.865	8.4	3.0	13.7	13.0	8.4	17.5
2	09-10-24	100	0.701	7.0	0.0964	13.8	0.728	0.591	0.865	8.3	2.9	13.7	12.9	8.5	17.4
3	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.8	8.0	17.6
4	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.5	7.3	17.8
5	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
6	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
7	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	12.4	7.3	17.4
8	02-04-25	100	0.733	3.1	0.1030	14.1	0.727	0.585	0.869	7.6	1.8	13.4	12.2	7.9	16.4
9	03-04-25	100	0.754	10.4	0.1103	14.6	0.729	0.588	0.871	7.7	1.8	13.6	12.3	8.0	16.7
10	04-08-25	100	0.746	7.8	0.0855	11.5	0.733	0.593	0.873	7.7	1.8	13.7	12.3	8.0	16.7
11	05-06-25	100	0.783	5.2	0.1099	14.0	0.732	0.593	0.871	7.8	2.0	13.6	12.5	8.1	16.8
12	06-03-25	100	0.920	4.4	0.1429	15.5	0.736	0.580	0.893	7.7	1.7	13.6	12.7	8.2	17.2
13	07-08-25	100	0.983	12.5	0.1150	11.7	0.754	0.570	0.938	7.9	1.5	14.2	12.7	8.1	17.2
14	08-05-25	100	0.959	2.7	0.0941	9.8	0.763	0.558	0.968	7.7	1.0	14.4	12.6	7.9	17.2
15	09-09-25	100	0.835	14.0	0.1126	13.5	0.767	0.559	0.975	7.8	0.9	14.7	12.3	8.4	16.2
16	10-07-25	100	0.774	5.0	0.0646	8.3	0.762	0.560	0.964	7.5	0.6	14.4	12.2	7.9	16.5
17	11-04-25	100	0.914	2.9	0.1313	14.4	0.775	0.569	0.981	7.3	0.1	14.4	12.2	7.8	16.6
18	12-02-25	100	0.629	5.0	0.0623	9.9	0.772	0.559	0.985	6.9	0.0	13.8	12.1	7.6	16.6
19	01-06-26	100	0.914	5.0	0.0736	8.1	0.783	0.564	1.002	6.8	-0.2	13.7	11.9	7.0	16.7
20	02-10-26	100	0.916	6.5	0.1204	13.1	0.795	0.574	1.016	6.6	-0.2	13.5	11.9	7.0	16.7

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.

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: **137**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2447				
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:	02-03-26	*Artemia* CHM number:	CHM1385
Hatch dates and times:	02-09-26 1300 TO 02-10-26 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.26 S.U. Temperature = 24.3 °C	Date / Time in oven:	02-17-26 0615
		*Initial oven temperature:	60° C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	02-18-26 0615
		*Final oven temperature:	60° C
		Total drying time:	24 HOURS

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	02-10-26	0505	JK	1500	JK	0705	JK	02-04-26B
1	02-11-26	0500	JK	1130	JK	0700	JK	↓
2	02-12-26	0500	JK	1130	JK	0745	JK	02-09-26 A
3	02-13-26	0500	JK	1100	JK	0730	JK	↓
4	02-14-26	0600	JK	1250	JK	0800	JK	02-09-26 B
5	02-15-26	0600	JK	1115	JK	0800	JK	↓
6	02-16-26	0500	JK	1100	JK	0700	JK	
7	02-17-26					0530	JK	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria
% Mortality:	0%	≤ 20%
Average weight per initial larvae:	0.916	
Average weight per surviving larvae:	0.916	≥ 0.25 mg/larvae

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	749.1
NOEC (mg/L KCl)	600
LOEC (mg/L KCl)	750
ChV (mg/L KCl)	670.8
IC_{25} (mg/L KCl)	643.1

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **137**

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: JP Date: 01-20-26	4.88	13.89	13.55	13.91	12.43	14.39	10.29	12.55	10.58	15.23	14.92	12.60
*B = Pan + Larvae weight (mg) Analyst: JH Date: 02-19-26	18.39	22.70	23.13	23.66	21.91	24.81	22.22	23.43	19.48	24.16	23.67	21.97
C = Larvae weight (mg) = B – A Analyst: JP	8.51	8.81	9.58	9.75	9.43	9.92	11.93	10.88	8.90	8.93	8.75	9.37
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JP	0.851	0.881	0.958	0.975	0.943	0.992	1.193	1.088	0.890	0.893	0.875	0.937
Average weight per initial number of larvae (mg)	0.916				1.0541		-15.0%		0.899		1.9%	
Percent reduction from control (%)												

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **137**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	4 bd	5 sd	4 bd	4 bd
2	10	10	10	10	9 id	10	10	9 id	4	4 id	4	4
3	10	10	10	10	9	9 id	9 id	9	4	3 id	3 id	3 id
4	10	10	9 id	10	9	8 id	9	8 id	2 2d	3	3	2 id
5	9 id	10	9	9 id	6 3d	5 3d	5 4d	5 3d	1 id	1 2d	1 2d	2
6	9	10	9	9	6	5	5	5	1	1	1	1 id
7	9	10	9	9	5 id	5	5	5	1	1	1	1
*A = Pan weight (mg) Tray color code: Turquoise Analyst: JP Date: 01-20-26	9.95	13.86	13.30	14.77	13.49	14.03	10.24	12.60	10.25	12.60	12.80	15.24
*B = Pan + Larvae weight (mg) Analyst: JP Date: 02.19.26	17.23	22.98	21.22	23.27	19.39	18.62	15.60	17.95	11.62	13.82	13.63	16.24
C = Larvae weight (mg) = B – A Analyst: JP	7.38	9.12	7.92	8.50	5.90	4.59	5.36	5.35	1.37	1.22	0.83	1.00
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JP	0.738	0.912	0.792	0.850	0.590	0.459	0.536	0.535	0.137	0.122	0.083	0.100
Average weight per initial number of larvae (mg)	0.823				~~0.525~~ 0.530				0.111			
Percent reduction from control (%)		10.2%			2-19-26	42.2%				87.9%		

*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: **137**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	2 8d	2 8d	2 8d	1 9d
2	2	1 1d	0 2d	0 1d
3	1 1d	1	0	0
4	1	0 1d	0	0
5	1	0	0	0
6	1	0	0	0
7	1	0	0	0
*A = Pan weight (mg) Tray color code: Turquoise Analyst: ZP Date: 01-20-26	10.46	14.42	14.19	14.47
*B = Pan + Larvae weight (mg) Analyst: M Date: 02-19-26	11.32			
C = Larvae weight (mg) = B – A Analyst: ZP	0.86			
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: ZP	0.086	0	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.022		97.7%	

02-19-26 M

*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: 137

Test dates: February 10-17, 2026

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	9.88	18.39	8.51	0.851	0.916	6.5	0.851	100.0	0.916	6.5	Not applicable
	B	10	10	13.89	22.70	8.81	0.881			0.881				
	C	10	10	13.55	23.13	9.58	0.958			0.958				
	D	10	10	13.91	23.66	9.75	0.975			0.975				
300	E	10	10	12.48	21.91	9.43	0.943	1.054	10.5	0.943	100.0	1.054	10.5	-15.0
	F	10	10	14.89	24.81	9.92	0.992			0.992				
	G	10	10	10.29	22.22	11.93	1.193			1.193				
	H	10	10	12.55	23.43	10.88	1.088			1.088				
450	I	10	10	10.58	19.48	8.90	0.890	0.899	3.0	0.890	100.0	0.899	3.0	1.9
	J	10	10	15.23	24.16	8.93	0.893			0.893				
	K	10	10	14.92	23.67	8.75	0.875			0.875				
	L	10	10	12.60	21.97	9.37	0.937			0.937				
600	M	10	9	9.85	17.23	7.38	0.820	0.889	6.0	0.738	92.5	0.823	9.1	10.2
	N	10	10	13.86	22.98	9.12	0.912			0.912				
	O	10	9	13.30	21.22	7.92	0.880			0.792				
	P	10	9	14.77	23.27	8.50	0.944			0.850				
750	Q	10	5	13.49	19.39	5.90	1.180	1.060	10.2	0.590	50.0	0.530	10.2	42.2
	R	10	5	14.03	18.62	4.59	0.918			0.459				
	S	10	5	10.24	15.60	5.36	1.072			0.536				
	T	10	5	12.60	17.95	5.35	1.070			0.535				
900	U	10	1	10.25	11.62	1.37	1.370	1.105	21.5	0.137	10.0	0.111	21.5	87.9
	V	10	1	12.60	13.82	1.22	1.220			0.122				
	W	10	1	12.80	13.63	0.83	0.830			0.083				
	X	10	1	15.24	16.24	1.00	1.000			0.100				
1050	Y	10	1	10.46	11.32	0.86	0.860	0.287	173.2	0.086	2.5	0.022	200.0	97.7
	Z	10	0	14.42	0.00	0.00	0.000			0.000				
	AA	10	0	14.19	0.00	0.00	0.000			0.000				
	BB	10	0	14.47	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.1204 MSD = Minimum Significant Difference

PMSD: 13.1 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.

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	2/10/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	2/17/2026	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	0.9000	0.9000
750	0.5000	0.5000	0.5000	0.5000
900	0.1000	0.1000	0.1000	0.1000
1050	0.1000	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	0.9250	0.9250	1.2898	1.2490	1.4120	6.318	4	12.00	10.00	3	40
*750	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	4	10.00	10.00	20	40
*900	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	4	10.00	10.00	36	40
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

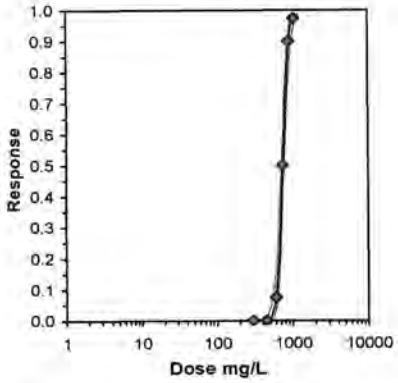
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.58279	0.896	2.2845	6.47308
Equality of variance cannot be confirmed				

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

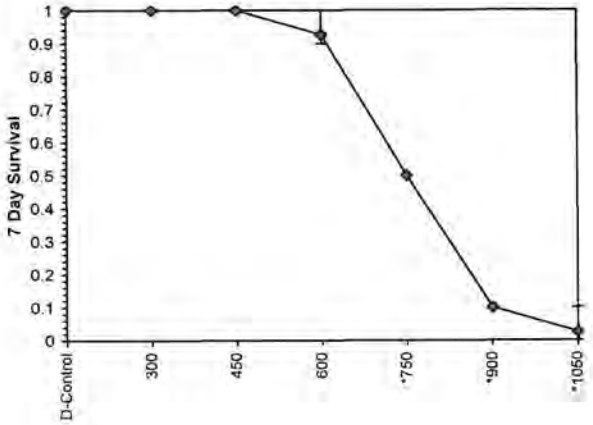
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.7859	1.83926	11.1809	18.3908	0	0.4332	9.48773	0.97967	2.87453	0.06763	3
Intercept	-37.502	5.30813	-47.906	-27.098							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	521.428	456.465	567.227
EC05	3.355	579.81	523.773	619.483
EC10	3.718	613.559	563.148	649.825
EC15	3.964	637.433	591.043	671.508
EC20	4.158	657.067	613.91	689.576
EC25	4.326	674.394	633.955	705.772
EC40	4.747	720.106	685.629	750.24
EC50	5.000	749.084	716.916	780.288
EC60	5.253	779.229	747.846	813.478
EC75	5.674	832.047	798.055	876.377
EC80	5.842	853.987	817.681	904.015
EC85	6.036	880.292	840.503	938.074
EC90	6.282	914.544	869.329	983.656
EC95	6.645	967.778	912.706	1056.68
EC99	7.326	1076.13	997.645	1211.45



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Stanfield

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	2/10/2026	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	2/17/2026	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:					

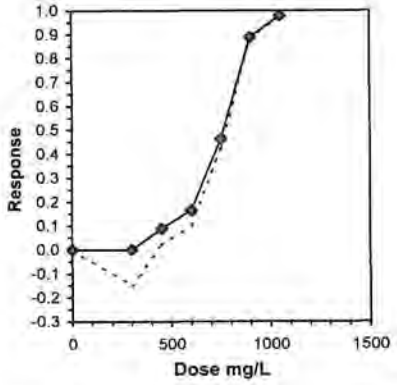
Conc-mg/L	1	2	3	4
D-Control	0.8510	0.8810	0.9580	0.9750
300	0.9430	0.9920	1.1930	1.0880
450	0.8900	0.8930	0.8750	0.9370
600	0.7380	0.9120	0.7920	0.8500
750	0.5900	0.4590	0.5360	0.5350
900	0.1370	0.1220	0.0830	0.1000
1050	0.0860	0.0000	0.0000	0.0000

			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	0.9163	1.0000	0.9163	0.8510	0.9750	6.516	4				0.9851	1.0000
300	1.0540	1.1503	1.0540	0.9430	1.1930	10.485	4	-2.621	2.290	0.1204	0.9851	1.0000
450	0.8988	0.9809	0.8988	0.8750	0.9370	2.969	4	0.333	2.290	0.1204	0.8988	0.9123
600	0.8230	0.8982	0.8230	0.7380	0.9120	9.102	4	1.774	2.290	0.1204	0.8230	0.8354
750	0.5300	0.5784	0.5300	0.4590	0.5900	10.162	4				0.5300	0.5380
900	0.1105	0.1206	0.1105	0.0830	0.1370	21.549	4				0.1105	0.1122
1050	0.0215	0.0235	0.0215	0.0000	0.0860	200.000	4				0.0215	0.0218

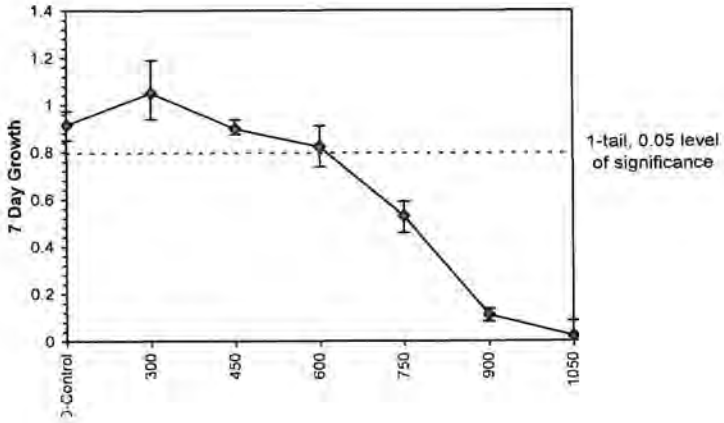
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.98263		0.844		0.30986	-0.1669
Bartlett's Test indicates equal variances (p = 0.22)					4.42116		11.3449			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	600	>600			0.12037	0.13137	0.03706	0.00553	0.00657	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	385.54	33.25	332.15	541.68	1.0722
IC10	474.03	55.02	362.53	690.59	0.5543
IC15	571.57	45.01	401.89	665.70	-0.7008
IC20	617.87	22.21	522.82	671.35	-0.9593
IC25	643.08	15.76	597.26	688.03	-0.2319
IC40	718.73	12.82	682.73	759.67	0.0539
IC50	763.39	9.34	732.69	786.37	-0.3930



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **137**

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	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.82	7.79	7.78	7.77	7.83	7.79
	Dissolved oxygen (mg/L)	8.4	7.9	8.4	7.9	8.5	7.8
	Conductivity (µmhos/cm)	295		302		295	
	Alkalinity (mg $CaCO_3$/L)	63				60	
	Hardness (mg $CaCO_3$/L)	86				84	
	Temperature (°C)	24.9	24.8	24.8	25.1	24.8	24.8
300 mg KCl/L	pH (S.U.)	7.95	7.76	7.88	7.76	7.97	7.76
	Dissolved oxygen (mg/L)	8.5	7.8	8.2	7.9	8.4	7.8
	Conductivity (µmhos/cm)	807		810		814	
	Temperature (°C)	25.0	25.0	24.8	25.0	24.9	24.6
450 mg KCl/L	pH (S.U.)	7.96	7.76	~~7.85~~ 7.91	7.77	7.98	7.76
	Dissolved oxygen (mg/L)	8.5	7.8	8.3	7.9	8.5	7.7
	Conductivity (µmhos/cm)	1050		1060		1040	
	Temperature (°C)	24.8	25.0	24.7	24.8	24.9	24.6
600 mg KCl/L	pH (S.U.)	7.96	7.76	~~7.85~~ 7.91	7.78	7.98	7.76
	Dissolved oxygen (mg/L)	8.6	7.8	8.3	7.8	8.5	7.5
	Conductivity (µmhos/cm)	1280		1300		1290	
	Temperature (°C)	25.0	25.0	24.7	24.8	24.7	24.7
750 mg KCl/L	pH (S.U.)	7.97	7.77	7.92	7.78	7.99	7.75
	Dissolved oxygen (mg/L)	8.6	7.8	8.3	7.8	8.5	7.6
	Conductivity (µmhos/cm)	1500		1540		1510	
	Temperature (°C)	25.0	24.9	24.7	24.8	24.7	24.7
900 mg KCl/L	pH (S.U.)	7.98	7.78	7.92	7.78	7.99	7.77
	Dissolved oxygen (mg/L)	8.6	7.8	8.3	7.8	8.5	7.8
	Conductivity (µmhos/cm)	1750		1780		1760	
	Temperature (°C)	25.0	24.7	24.8	25.1	24.8	24.9
1050 mg KCl/L	pH (S.U.)	7.98	7.79	7.91	7.79	7.99	7.78
	Dissolved oxygen (mg/L)	8.5	7.9	8.3	7.9	8.5	7.7
	Conductivity (µmhos/cm)	2030		2050		2030	
	Temperature (°C)	25.1	24.7	24.7	24.8	24.7	24.9
		Initial	Final	Initial	Final	Initial	Final

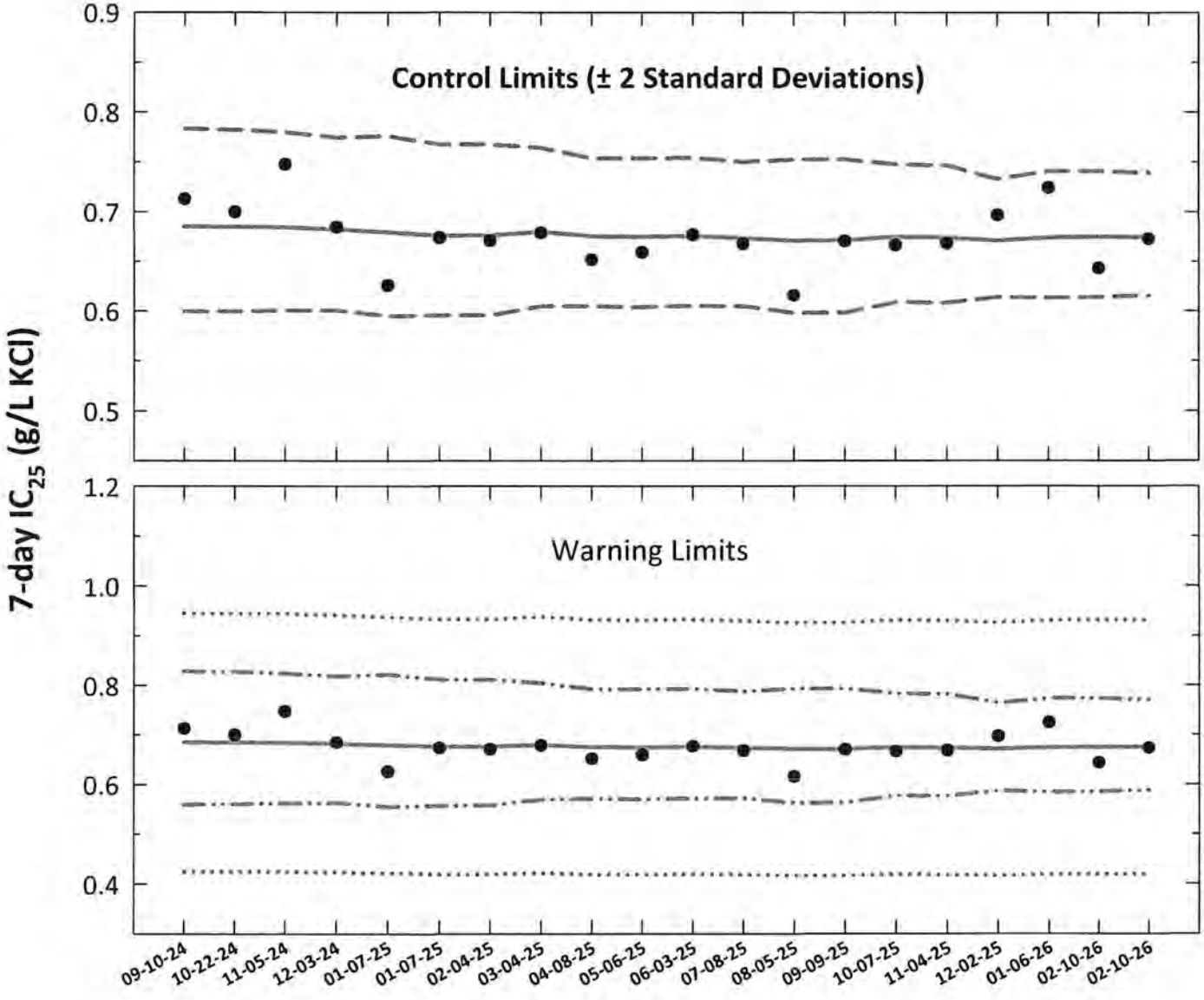
Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **137**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	XL
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.85	7.75	7.79	7.71	7.81	7.91	7.87	7.44
	Dissolved oxygen (mg/L)	8.5	7.8	8.6	7.7	8.6	8.2	8.4	5.8
	Conductivity (µmhos/cm)	297		285		277		306	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)			88					
	Temperature (°C)	24.8	25.0	24.7	24.9	24.8	24.8	24.7	25.0
300 mg KCl/L	pH (S.U.)	7.97	7.74	8.03	7.73	8.02	7.88	7.99	7.41
	Dissolved oxygen (mg/L)	8.4	7.9	8.5	7.6	8.4	8.1	8.3	5.6
	Conductivity (µmhos/cm)	811		776		763		827	
	Temperature (°C)	24.7	24.7	24.8	24.7	24.9	25.0	24.7	24.8
450 mg KCl/L	pH (S.U.)	7.98	7.76	8.02	7.73	8.04	7.87	8.01	7.42
	Dissolved oxygen (mg/L)	8.4	7.9	8.6	7.4	8.5	8.1	8.3	5.6
	Conductivity (µmhos/cm)	1040		1010		998		1050	
	Temperature (°C)	24.7	24.7	24.8	24.7	25.0	24.7	24.8	24.9
600 mg KCl/L	pH (S.U.)	7.98	7.75	8.02	7.73	8.05	7.86	8.01	7.42
	Dissolved oxygen (mg/L)	8.4	7.7	8.6	7.1	8.5	8.1	8.3	5.6
	Conductivity (µmhos/cm)	1290		1230		1230		1300	
	Temperature (°C)	24.7	24.9	24.9	24.9	24.9	24.7	24.8	25.1
750 mg KCl/L	pH (S.U.)	7.98	7.75	8.02	7.71	8.05	7.86	8.01	7.42
	Dissolved oxygen (mg/L)	8.4	7.8	8.5	7.2	8.4	8.0	8.4	5.6
	Conductivity (µmhos/cm)	1520		1500		1460		1540	
	Temperature (°C)	24.9	24.6	24.9	25.0	24.9	25.0	24.9	24.7
900 mg KCl/L	pH (S.U.)	7.99	7.77	8.02	7.71	8.04	7.85	8.01	7.43
	Dissolved oxygen (mg/L)	8.4	7.9	8.6	7.4	8.5	8.0	8.4	6.0
	Conductivity (µmhos/cm)	1770		1720		1670		1770	
	Temperature (°C)	24.9	24.9	25.0	~~7.72~~ 24.8	24.9	24.8	24.8	25.1
1050 mg KCl/L	pH (S.U.)	7.99	7.83	8.02	7.72	8.06	7.88	8.01	7.44
	Dissolved oxygen (mg/L)	8.4	7.9	8.5	7.5	8.5	8.1	8.4	6.0
	Conductivity (µmhos/cm)	2040		1940		1930		2050	
	Temperature (°C)	25.0	24.5	25.0	24.8	25.0	24.8	24.8	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

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)

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	09-10-24	0.7128	-0.1470	-0.1642	0.0290	0.6852	0.5994	0.7832	0.5600	0.8283	0.4248	0.9456
2	10-22-24	0.6997	-0.1551	-0.1645	0.0289	0.6847	0.5994	0.7822	0.5601	0.8271	0.4245	0.9449
3	11-05-24	0.7473	-0.1265	-0.1648	0.0284	0.6842	0.6004	0.7797	0.5617	0.8238	0.4242	0.9442
4	12-03-24	0.6844	-0.1647	-0.1662	0.0276	0.6820	0.6006	0.7745	0.5626	0.8176	0.4228	0.9411
5	01-07-25	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946	0.7757	0.5547	0.8213	0.4211	0.9372
6	01-07-25	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955	0.7675	0.5569	0.8113	0.4191	0.9329
7	02-04-25	0.6706	-0.1735	-0.1699	0.0275	0.6762	0.5956	0.7676	0.5571	0.8114	0.4192	0.9331
8	03-04-25	0.6786	-0.1684	-0.1676	0.0254	0.6798	0.6047	0.7643	0.5693	0.8041	0.4215	0.9381
9	04-08-25	0.6516	-0.1860	-0.1705	0.0239	0.6753	0.6048	0.7540	0.5708	0.7919	0.4187	0.9319
10	05-06-25	0.6589	-0.1812	-0.1709	0.0241	0.6746	0.6039	0.7537	0.5697	0.7918	0.4183	0.9310
11	06-03-25	0.6768	-0.1695	-0.1703	0.0239	0.6757	0.6053	0.7543	0.5715	0.7920	0.4189	0.9324
12	07-08-25	0.6674	-0.1756	-0.1716	0.0234	0.6736	0.6048	0.7501	0.5715	0.7872	0.4176	0.9295
13	08-05-25	0.6156	-0.2107	-0.1734	0.0249	0.6709	0.5981	0.7525	0.5623	0.7926	0.4159	0.9258
14	09-09-25	0.6703	-0.1738	-0.1731	0.0249	0.6712	0.5985	0.7529	0.5628	0.7928	0.4162	0.9263
15	10-07-25	0.6661	-0.1765	-0.1709	0.0223	0.6747	0.6087	0.7477	0.5769	0.7829	0.4183	0.9310
16	11-04-25	0.6681	-0.1752	-0.1715	0.0223	0.6738	0.6081	0.7466	0.5763	0.7819	0.4177	0.9298
17	12-02-25	0.6967	-0.1570	-0.1733	0.0192	0.6710	0.6141	0.7331	0.5862	0.7636	0.4160	0.9260
18	01-06-26	0.7242	-0.1401	-0.1713	0.0205	0.6741	0.6133	0.7409	0.5839	0.7732	0.4179	0.9302
19	02-10-26	0.6431	-0.1917	-0.1725	0.0210	0.6722	0.6103	0.7405	0.5800	0.7738	0.4168	0.9277
20	02-10-26	0.6727	-0.1722	-0.1710	0.0199	0.6745	0.6156	0.7391	0.5871	0.7703	0.4182	0.9308

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.

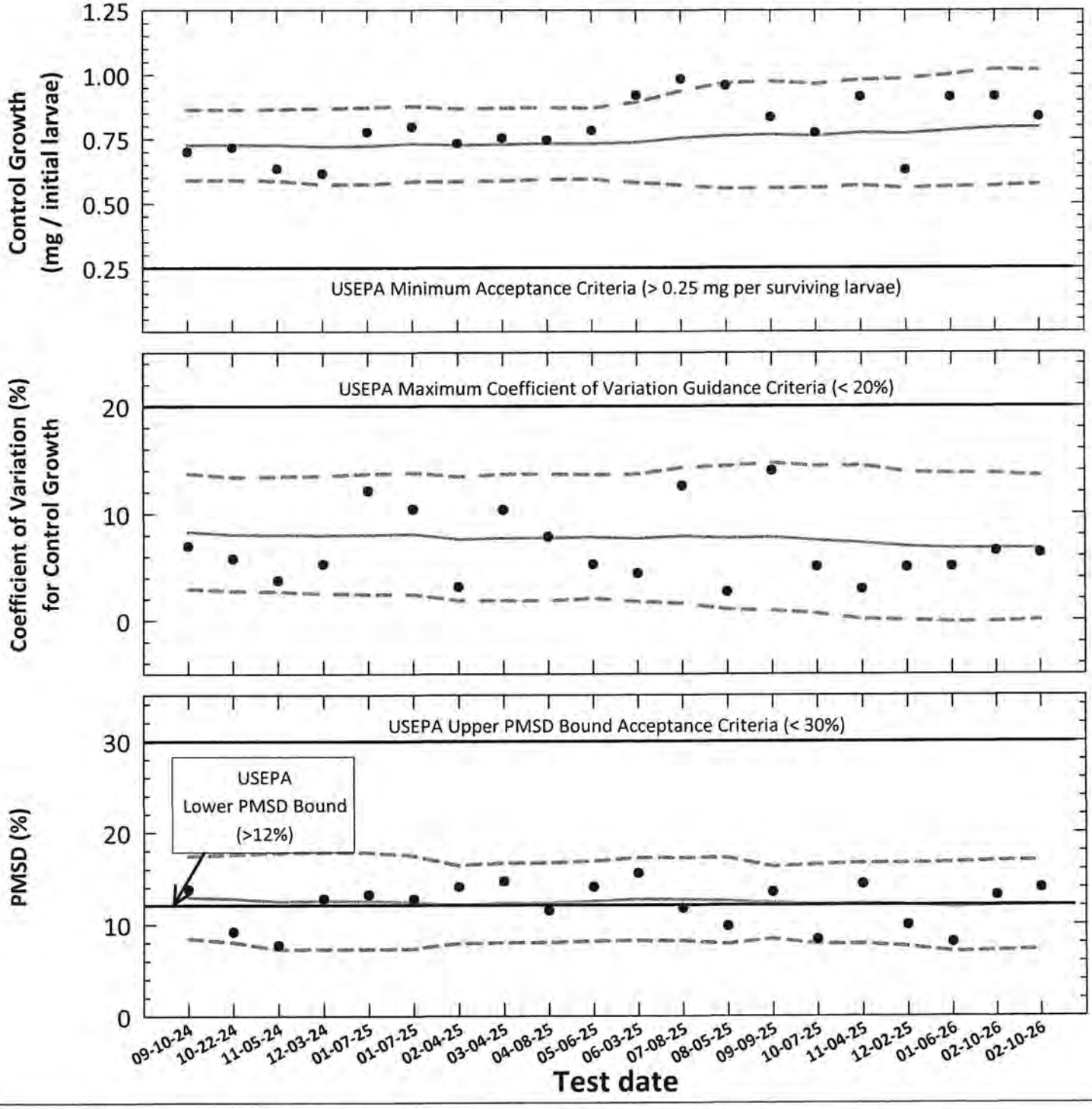
Entered and Reviewed by Jim Sumner

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			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	09-10-24	100	0.701	7.0	0.0964	13.8	0.728	0.591	0.865	8.3	2.9	13.7	12.9	8.5	17.4
2	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.8	8.0	17.6
3	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.5	7.3	17.8
4	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
5	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
6	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	12.4	7.3	17.4
7	02-04-25	100	0.733	3.1	0.1030	14.1	0.727	0.585	0.869	7.6	1.8	13.4	12.2	7.9	16.4
8	03-04-25	100	0.754	10.4	0.1103	14.6	0.729	0.588	0.871	7.7	1.8	13.6	12.3	8.0	16.7
9	04-08-25	100	0.746	7.8	0.0855	11.5	0.733	0.593	0.873	7.7	1.8	13.7	12.3	8.0	16.7
10	05-06-25	100	0.783	5.2	0.1099	14.0	0.732	0.593	0.871	7.8	2.0	13.6	12.5	8.1	16.8
11	06-03-25	100	0.920	4.4	0.1429	15.5	0.736	0.580	0.893	7.7	1.7	13.6	12.7	8.2	17.2
12	07-08-25	100	0.983	12.5	0.1150	11.7	0.754	0.570	0.938	7.9	1.5	14.2	12.7	8.1	17.2
13	08-05-25	100	0.959	2.7	0.0941	9.8	0.763	0.558	0.968	7.7	1.0	14.4	12.6	7.9	17.2
14	09-09-25	100	0.835	14.0	0.1126	13.5	0.767	0.559	0.975	7.8	0.9	14.7	12.3	8.4	16.2
15	10-07-25	100	0.774	5.0	0.0646	8.3	0.762	0.560	0.964	7.5	0.6	14.4	12.2	7.9	16.5
16	11-04-25	100	0.914	2.9	0.1313	14.4	0.775	0.569	0.981	7.3	0.1	14.4	12.2	7.8	16.6
17	12-02-25	100	0.629	5.0	0.0623	9.9	0.772	0.559	0.985	6.9	0.0	13.8	12.1	7.6	16.6
18	01-06-26	100	0.914	5.0	0.0736	8.1	0.783	0.564	1.002	6.8	-0.2	13.7	11.9	7.0	16.7
19	02-10-26	100	0.916	6.5	0.1204	13.1	0.795	0.574	1.016	6.6	-0.2	13.5	11.9	7.0	16.7
20	02-10-26	100	0.838	6.3	0.1170	14.0	0.797	0.575	1.019	6.8	0.0	13.5	12.1	7.3	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: **138 – New Artemia**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2447				
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:	BWC
Age:	< 24-hours old	Incubator number and shelf location:	7C
Spawn date:	02·03·26	*Artemia* CHM number:	1432
Hatch dates and times:	02·09·26 1300 TO 02·10·26 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.26 S.U. Temperature = 24.3 °C	Date / Time in oven:	02·17·26 0615
		*Initial oven temperature:	60·C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	02·18·26 0615
		*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	02-10-26	0505	JK	1500	JK	0705	JK	02·04·26 B
1	02-11-26	0500	JK	1130	JK	0700	JK	↓
2	02-12-26	0500	JK	1130	JK	0745	JK	02·09·26 A
3	02-13-26	0500	JK	1100	JK	0730	JK	↓
4	02-14-26	0600	JK	1250	JK	0800	JK	02·09·26 B
5	02-15-26	0600	JK	02·15·26 1115	JK	0800	JK	↓
6	02-16-26	0500	JK	1600	JK	0700	JK	
7	02-17-26					0544	JK	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria
% Mortality:	0%	≤ 20%
Average weight per initial larvae:	0.838	
Average weight per surviving larvae:	0.838	≥ 0.25 mg/larvae

Summary of test endpoints:	
7-day LC_{50} (mg/L KCl)	750.2
NOEC (mg/L KCl)	600
LOEC (mg/L KCl)	750
ChV (mg/L KCl)	670.8
IC_{25} (mg/L KCl)	672.7

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **138 – 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: light pink Analyst: JO Date: 01-20-26	13.07	14.33	13.09	12.49	13.63	10.69	9.19	12.87	10.28	15.71	9.941	13.42
*B = Pan + Larvae weight (mg) Analyst: JO Date: 02-19-26	21.19	22.10	21.82	21.39	23.02	20.80	19.72	21.64	20.24	26.00	19.84	22.96
C = Larvae weight (mg) = B – A Analyst: JO	8.12	7.77	8.74	8.90	9.39	10.11	10.53	8.97	9.96	10.29	9.90	9.54
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JO	0.812	0.777	0.874	0.890	0.939	1.011	1.053	0.897	0.996	1.029	0.990	0.954
Average weight per initial number of larvae (mg)	0.838				0.975		-16.3%		0.992		-18.4%	
Percent reduction from control (%)												

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **138 – 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	10	10	10	10	4 6d	4 6d	4 6d	5 Sd
2	10	10	10	10	10	10	10	10	2 2d	4	4	4 1d
3	10	10	10	10	10	10	10	9 1d	2	3 1d	4	4
4	9 1d	10	10	10	8 1d	8 1d	8 1d	8 1d	2	3	3 1d	3 1d
5	9	10	10	10	6 2d	6 2d	6 2d	6 2d	1 1d	2 1d	2 1d	1 2d
6	9	10	10	10	6	6	6	6	1	1 1d	1 1d	1
7	9	10	9 1d	10	6	5 1d	5 1d	5 1d	1	1	1	0 1d
*A = Pan weight (mg) Tray color code: light pink Analyst: JP Date: 01-19-26	14.25	15.16	14.24	15.41	11.07	11.63	13.32	11.74	11.84	13.50	12.12	13.66
*B = Pan + Larvae weight (mg) Analyst: JP Date: 02-19-26	22.00	24.00	22.76	25.79	19.68	16.87	17.96	16.40	12.86	14.53	12.92	
C = Larvae weight (mg) = B – A Analyst: JP	7.75	8.84	8.52	10.42 (10.33)	(8.61) 10.37	5.24	4.64	4.66	1.02	1.03	0.80	N 0.17
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JP	0.775	0.884	0.852	1.038	0.561	0.524	0.464	0.466	0.102	0.103	0.080	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.887		-5.8%		0.524		39.9%		0.071		91.5%	

*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:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **138 – New Artemia**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	1 9d	1 9d	2 8d	2 8d
2	0 1d	0 1d	1 1d	1 1d
3	0	0	0 1d	1
4	0	0	0	1
5	0	0	0	1
6	0	0	0	0 1d
7	0	0	0	0
*A = Pan weight (mg) Tray color code: light pink Analyst: JP Date: 01-22-26	10.22	15.53	12.55	12.53
*B = Pan + Larvae weight (mg) Analyst: _____ Date: _____				
C = Larvae weight (mg) = B – A Analyst: _____				JP 02-16-26
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: _____	0	0	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0		100%	

*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:

ETS
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: 138 - New Artemia

Test dates: February 10-17, 2026

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	13.07	21.19	8.12	0.812	0.838	6.3	0.812	100.0	0.838	6.3	Not applicable
	B	10	10	14.33	22.10	7.77	0.777			0.777				
	C	10	10	13.08	21.82	8.74	0.874			0.874				
	D	10	10	12.49	21.39	8.90	0.890			0.890				
300	E	10	10	13.63	23.02	9.39	0.939	0.975	7.2	0.939	100.0	0.975	7.2	-16.3
	F	10	10	10.69	20.80	10.11	1.011			1.011				
	G	10	10	9.19	19.72	10.53	1.053			1.053				
	H	10	10	12.87	21.84	8.97	0.897			0.897				
450	I	10	10	10.28	20.24	9.96	0.996	0.992	3.1	0.996	100.0	0.992	3.1	-18.4
	J	10	10	15.71	26.00	10.29	1.029			1.029				
	K	10	10	9.94	19.84	9.90	0.990			0.990				
	L	10	10	13.42	22.96	9.54	0.954			0.954				
600	M	10	9	14.25	22.00	7.75	0.861	0.932	8.5	0.775	95.0	0.887	12.4	-5.8
	N	10	10	15.16	24.00	8.84	0.884			0.884				
	O	10	9	14.24	22.76	8.52	0.947			0.852				
	P	10	10	15.41	25.79	10.38	1.038			1.038				
750	Q	10	6	14.07	19.68	5.61	0.935	0.961	6.1	0.561	52.5	0.504	9.4	39.9
	R	10	5	11.63	16.87	5.24	1.048			0.524				
	S	10	5	13.32	17.96	4.64	0.928			0.464				
	T	10	5	11.74	16.40	4.66	0.932			0.466				
900	U	10	1	11.84	12.86	1.02	1.020	0.713	68.3	0.102	7.5	0.071	68.3	91.5
	V	10	1	13.50	14.53	1.03	1.030			0.103				
	W	10	1	12.12	12.92	0.80	0.800			0.080				
	X	10	0	13.66	0.00	0.00	0.000			0.000				
1050	Y	10	0	10.22	0.00	0.00	0.000	0.000	0.0	0.000	0.0	0.000	0.0	100.0
	Z	10	0	15.53	0.00	0.00	0.000			0.000				
	AA	10	0	12.55	0.00	0.00	0.000			0.000				
	BB	10	0	12.53	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.1171 MSD = Minimum Significant Difference

PMSD: 14.0 PMSD = Percent Minimum Significant Difference

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

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	2/10/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	2/17/2026	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	0.9000	1.0000
750	0.6000	0.5000	0.5000	0.5000
900	0.1000	0.1000	0.1000	0.0000
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	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.5250	0.5250	0.8106	0.7854	0.8861	6.210	4	10.00	10.00	19	40
*900	0.0750	0.0750	0.2810	0.1588	0.3218	28.997	4	10.00	10.00	37	40
1050	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

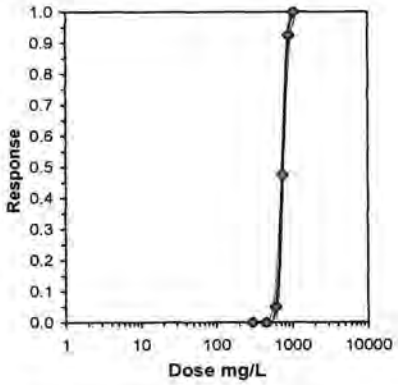
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.87978	0.884	-0.516	1.07413
Equality of variance cannot be confirmed				

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

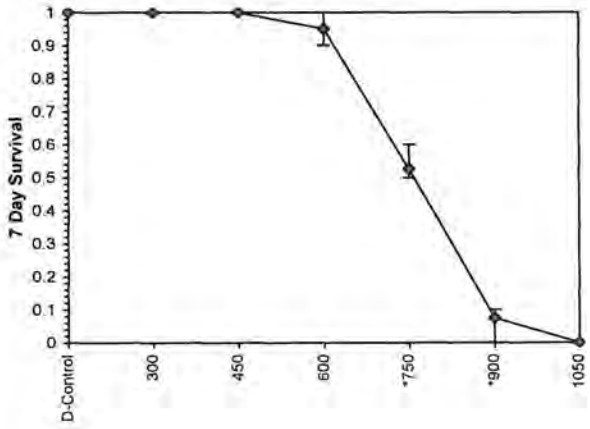
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	18.0372	2.38686	13.359	22.7155	0	0.36935	9.48773	0.98491	2.87518	0.05544	3
Intercept	-46.86	6.88392	-60.353	-33.368							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	557.445	495.136	599.63
EC05	3.355	608.113	555.397	644.199
EC10	3.718	636.981	590.006	669.817
EC15	3.964	657.228	614.253	688.026
EC20	4.158	673.777	633.964	703.148
EC25	4.326	688.307	651.122	716.667
EC40	4.747	726.325	694.839	753.634
EC50	5.000	750.199	720.959	778.488
EC60	5.253	774.859	746.534	805.806
EC75	5.674	817.657	787.553	857.167
EC80	5.842	835.289	803.419	879.571
EC85	6.036	856.323	821.754	907.049
EC90	6.282	883.542	844.741	943.6
EC95	6.645	925.484	878.995	1001.65
EC99	7.326	1009.61	945.036	1122.71



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	2/10/2026	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	2/17/2026	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:					

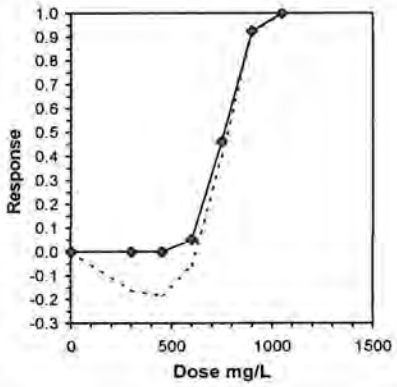
Conc-mg/L	1	2	3	4
D-Control	0.8120	0.7770	0.8740	0.8900
300	0.9390	1.0110	1.0530	0.8970
450	0.9960	1.0290	0.9900	0.9540
600	0.7750	0.8840	0.8510	1.0380
750	0.5610	0.5240	0.4640	0.4660
900	0.1020	0.1030	0.0800	0.0000
1050	0.0000	0.0000	0.0000	0.0000

			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	0.8383	1.0000	0.8383	0.7770	0.8900	6.311	4				0.9352	1.0000
300	0.9750	1.1631	0.9750	0.8970	1.0530	7.194	4	-2.677	2.290	0.1170	0.9352	1.0000
450	0.9923	1.1837	0.9923	0.9540	1.0290	3.097	4	-3.014	2.290	0.1170	0.9352	1.0000
600	0.8870	1.0582	0.8870	0.7750	1.0380	12.461	4	-0.954	2.290	0.1170	0.8870	0.9485
750	0.5038	0.6010	0.5038	0.4640	0.5610	9.376	4				0.5038	0.5387
900	0.0713	0.0850	0.0713	0.0000	0.1030	68.311	4				0.0713	0.0762
1050	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

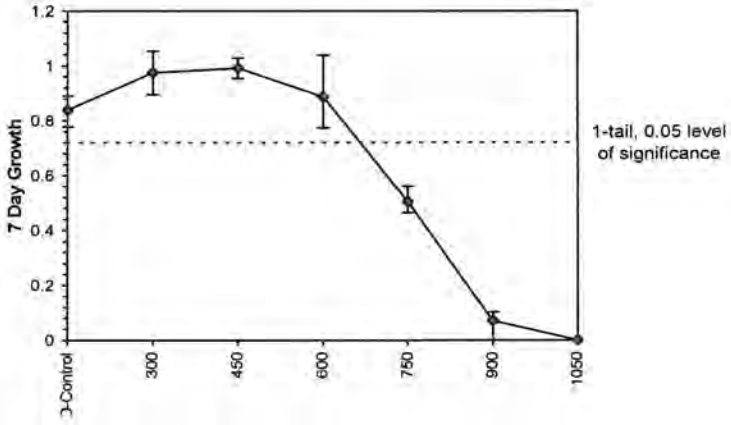
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.972		0.844		0.5425	0.76835
Bartlett's Test indicates equal variances (p = 0.25)					4.0618		11.3449			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	600	>600			0.11699	0.13957	0.0213	0.00522	0.03268	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	595.61	40.76	444.70	630.42	-0.5161
IC10	617.75	22.01	513.50	644.92	-1.5277
IC15	636.05	14.61	581.20	661.73	-1.1311
IC20	654.35	12.31	607.43	678.81	-0.8507
IC25	672.65	10.71	630.54	696.22	-0.7414
IC40	727.55	8.61	700.48	752.28	-0.0098
IC50	762.54	7.40	739.39	782.06	-0.0887



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKCICR Test Number: **138 – 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	XL	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.82	7.79	7.78	7.75	7.83	7.81
	Dissolved oxygen (mg/L)	8.4	7.9	8.4	7.9	8.5	7.9
	Conductivity (µmhos/cm)	295		302		295	
	Alkalinity (mg $CaCO_3$/L)	63				60	
	Hardness (mg $CaCO_3$/L)	86				84	
	Temperature (°C)	24.9	24.9	24.8	24.9	24.8	24.6
300 mg KCl/L	pH (S.U.)	7.95	7.78	7.88	7.73	7.97	7.78
	Dissolved oxygen (mg/L)	8.5	7.9	8.2	7.8	8.4	7.9
	Conductivity (µmhos/cm)	807		810		814	
	Temperature (°C)	25.1	24.6	24.9	25.1	24.9	24.6
450 mg KCl/L	pH (S.U.)	7.96	7.78	7.91	7.74	7.98	7.79
	Dissolved oxygen (mg/L)	8.5	7.7	8.3	7.9	8.5	7.8
	Conductivity (µmhos/cm)	1050		1060		1040	
	Temperature (°C)	25.0	24.9	24.9	24.8	24.9	24.9
600 mg KCl/L	pH (S.U.)	7.96	7.78	7.91	7.74	7.98	7.79
	Dissolved oxygen (mg/L)	8.6	7.7	8.3	7.9	8.5	7.9
	Conductivity (µmhos/cm)	1280		1300		1290	
	Temperature (°C)	25.0	25.0	25.0	24.8	24.7	24.5
750 mg KCl/L	pH (S.U.)	7.97	7.78	7.92	7.75	7.99	7.79
	Dissolved oxygen (mg/L)	8.6	7.8	8.3	7.9	8.5	7.9
	Conductivity (µmhos/cm)	1500		1540		1510	
	Temperature (°C)	25.0	25.0	24.8	25.0	24.7	24.5
900 mg KCl/L	pH (S.U.)	7.98	7.79	7.92	7.75	7.99	7.80
	Dissolved oxygen (mg/L)	8.6	7.8	8.3	7.9	8.5	7.9
	Conductivity (µmhos/cm)	1750		1780		1760	
	Temperature (°C)	25.2	25.2	24.8	25.0	24.7	24.6
1050 mg KCl/L	pH (S.U.)	7.98	7.80	7.91	7.76	7.99	7.81
	Dissolved oxygen (mg/L)	8.5	8.0	8.3	7.9	8.5	8.0
	Conductivity (µmhos/cm)	2030		2050		2030	
	Temperature (°C)	24.9	24.9	24.8	25.0	24.9	24.7
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas*

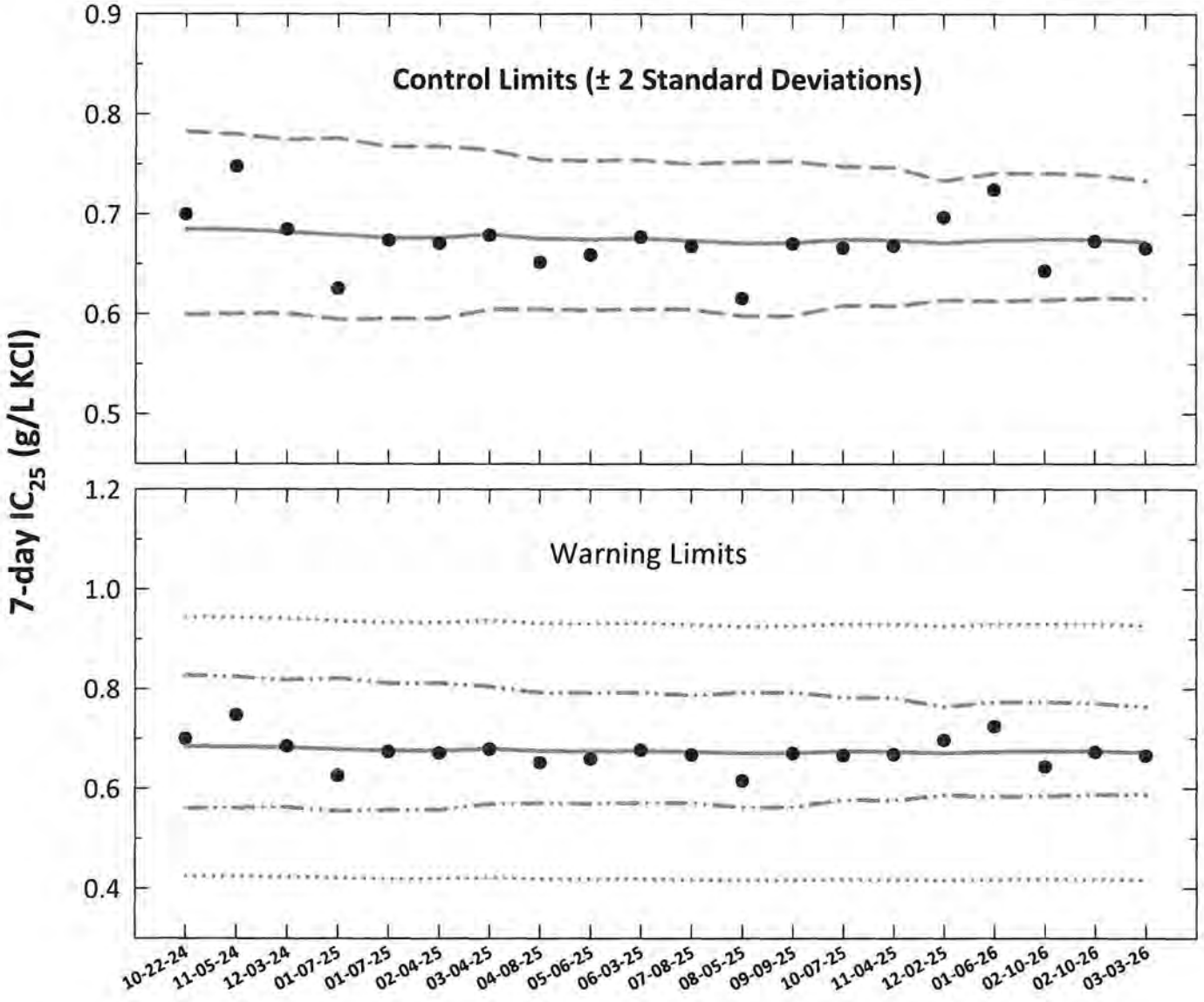
PpKClCR Test Number: **138 – New Artemia**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.85	7.76	7.79	7.70	7.81	7.85	7.87	7.45
	Dissolved oxygen (mg/L)	8.5	8.0	8.6	7.7	8.6	8.0	8.4	6.1
	Conductivity (µmhos/cm)	297		285		277		306	
	Alkalinity (mg $CaCO_3$/L)			63					
	Hardness (mg $CaCO_3$/L)		2-16-26 M	88					2-24 M
	Temperature (°C)	24.8	25.1	24.7	24.9	24.8	25.0	24.7	25.0
300 mg KCl/L	pH (S.U.)	7.97	7.75	8.03	7.69	~~8.28~~ (8.02)	7.85	7.99	7.43
	Dissolved oxygen (mg/L)	8.4	8.0	8.5	7.7	84	8.1	8.3	6.1
	Conductivity (µmhos/cm)	811		776		763		827	
	Temperature (°C)	24.9	24.7	24.8	25.1	24.9	24.7	24.7	24.7
450 mg KCl/L	pH (S.U.)	7.98	7.75	8.02	7.68	~~8.28~~ (8.04)	7.86	8.01	7.43
	Dissolved oxygen (mg/L)	8.4	7.7	8.6	7.1	8.5	8.1	8.3	5.9
	Conductivity (µmhos/cm)	1040		1010		998		1050	
	Temperature (°C)	24.9	24.7	24.8	24.9	24.9	24.7	24.8	24.7
600 mg KCl/L	pH (S.U.)	7.98	7.75	8.02	7.69	~~8.27~~ (8.05)	7.86	8.01	7.43
	Dissolved oxygen (mg/L)	8.4	7.4	8.6	7.4	8.5	8.1	8.3	5.8
	Conductivity (µmhos/cm)	1290		1230		1230		1300	
	Temperature (°C)	25.0	24.6	24.9	24.9	24.8	24.9	24.8	24.7
750 mg KCl/L	pH (S.U.)	7.98	7.82	8.02	7.69	~~8.25~~ (8.03)	7.86	8.01	7.44
	Dissolved oxygen (mg/L)	8.4	7.7	8.5	7.3	8.4	8.0	8.4	6.1
	Conductivity (µmhos/cm)	1520		1500		1460		1540	
	Temperature (°C)	24.7	24.8	24.7	24.7	24.8	24.9	24.8	24.9
900 mg KCl/L	pH (S.U.)	7.99	7.90	8.02	7.73	~~8.24~~ (8.06)	7.86	8.01	7.45
	Dissolved oxygen (mg/L)	8.4	7.7	8.6	7.3	8.5	8.1	8.4	5.9
	Conductivity (µmhos/cm)	1770		1720		1670		1770	
	Temperature (°C)	24.7	24.7	24.7	24.7	24.8	24.7	24.7	24.9
1050 mg KCl/L	pH (S.U.)	7.99	7.94	8.02	7.74	~~8.27~~ (8.06)	7.87	8.01	
	Dissolved oxygen (mg/L)	8.4	7.9	8.5	7.7	8.5	8.0	8.4	
	Conductivity (µmhos/cm)	2040		1940		1930		2050	2-24 M
	Temperature (°C)	24.7	24.7	24.9	24.7	24.9	25.1	24.7	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

* verified correct BSL 02.15.26

ETS
Environmental Testing Solutions, Inc.

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 $IC_{25} \pm S_{A.75}$ 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	10-22-24	0.6997	-0.1551	-0.1645	0.0289	0.6847	0.5994	0.7822	0.5601	0.8271	0.4245	0.9449
2	11-05-24	0.7473	-0.1265	-0.1648	0.0284	0.6842	0.6004	0.7797	0.5617	0.8238	0.4242	0.9442
3	12-03-24	0.6844	-0.1647	-0.1662	0.0276	0.6820	0.6006	0.7745	0.5626	0.8176	0.4228	0.9411
4	01-07-25	0.6255	-0.2038	-0.1680	0.0289	0.6792	0.5946	0.7757	0.5547	0.8213	0.4211	0.9372
5	01-07-25	0.6737	-0.1715	-0.1700	0.0276	0.6760	0.5955	0.7675	0.5569	0.8113	0.4191	0.9329
6	02-04-25	0.6706	-0.1735	-0.1699	0.0275	0.6762	0.5956	0.7676	0.5571	0.8114	0.4192	0.9331
7	03-04-25	0.6786	-0.1684	-0.1676	0.0254	0.6798	0.6047	0.7643	0.5693	0.8041	0.4215	0.9381
8	04-08-25	0.6516	-0.1860	-0.1705	0.0239	0.6753	0.6048	0.7540	0.5708	0.7919	0.4187	0.9319
9	05-06-25	0.6589	-0.1812	-0.1709	0.0241	0.6746	0.6039	0.7537	0.5697	0.7918	0.4183	0.9310
10	06-03-25	0.6768	-0.1695	-0.1703	0.0239	0.6757	0.6053	0.7543	0.5715	0.7920	0.4189	0.9324
11	07-08-25	0.6674	-0.1756	-0.1716	0.0234	0.6736	0.6048	0.7501	0.5715	0.7872	0.4176	0.9295
12	08-05-25	0.6156	-0.2107	-0.1734	0.0249	0.6709	0.5981	0.7525	0.5623	0.7926	0.4159	0.9258
13	09-09-25	0.6703	-0.1738	-0.1731	0.0249	0.6712	0.5985	0.7529	0.5628	0.7928	0.4162	0.9263
14	10-07-25	0.6661	-0.1765	-0.1709	0.0223	0.6747	0.6087	0.7477	0.5769	0.7829	0.4183	0.9310
15	11-04-25	0.6681	-0.1752	-0.1715	0.0223	0.6738	0.6081	0.7466	0.5763	0.7819	0.4177	0.9298
16	12-02-25	0.6967	-0.1570	-0.1733	0.0192	0.6710	0.6141	0.7331	0.5862	0.7636	0.4160	0.9260
17	01-06-26	0.7242	-0.1401	-0.1713	0.0205	0.6741	0.6133	0.7409	0.5839	0.7732	0.4179	0.9302
18	02-10-26	0.6431	-0.1917	-0.1709	0.0204	0.6746	0.6141	0.7411	0.5849	0.7732	0.4183	0.9310
19	02-10-26	0.6727	-0.1722	-0.1710	0.0199	0.6745	0.6156	0.7391	0.5871	0.7703	0.4182	0.9308
20	03-03-26	0.6656	-0.1768	-0.1725	0.0191	0.6722	0.6157	0.7339	0.5881	0.7640	0.4168	0.9277

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.

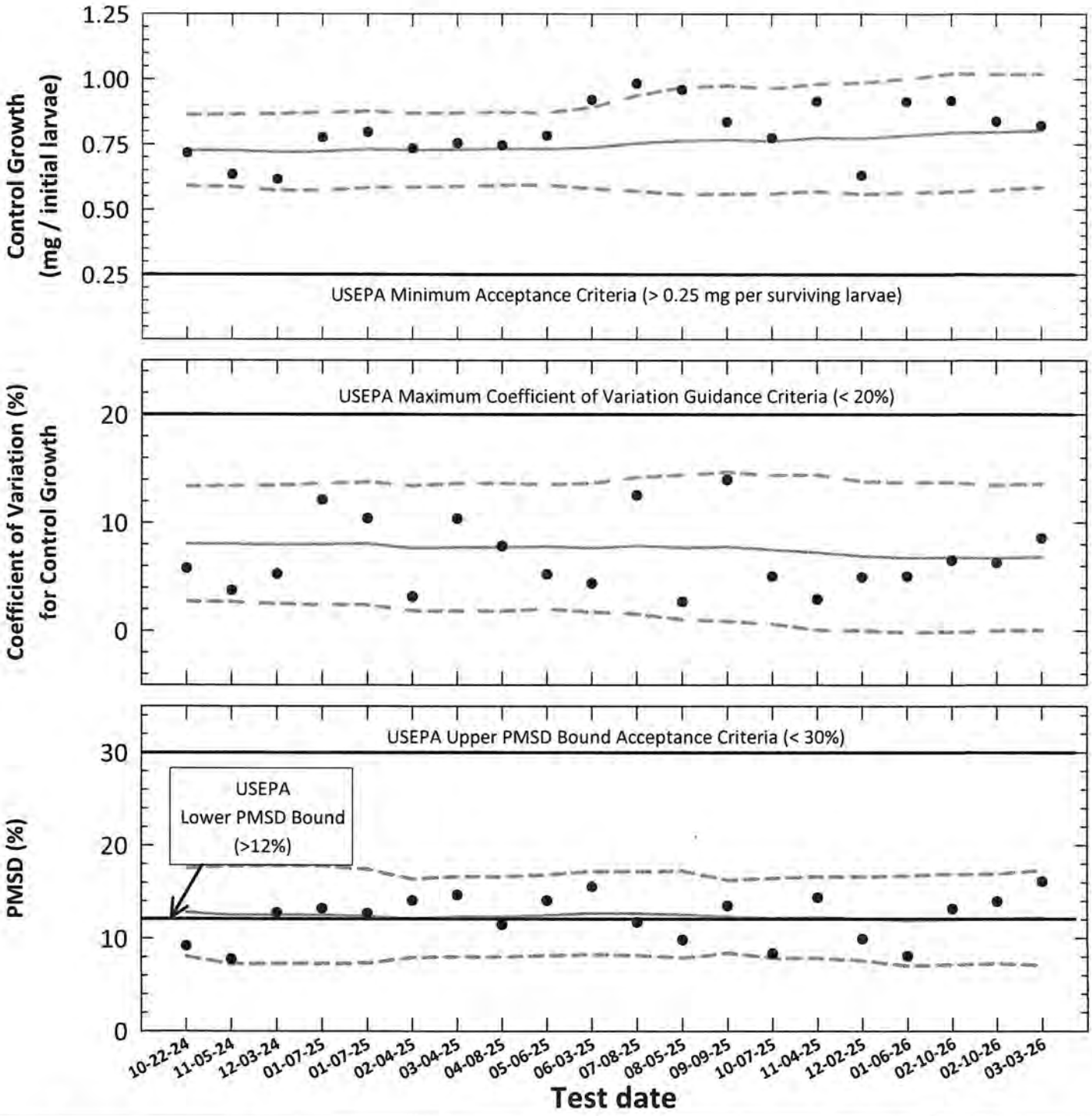
Entered and Reviewed by Jim Sumner

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			95% Confidence Interval			95% Confidence Interval			95% Confidence Interval	
		(%)	Mean (mg/initial larvae)	CV (%)	MSD	PMSD (%)	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S	CT	CT - 2S	CT + 2S
1	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.8	8.0	17.6
2	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.5	7.3	17.8
3	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	12.5	7.3	17.8
4	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	12.5	7.3	17.8
5	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	12.4	7.3	17.4
6	02-04-25	100	0.733	3.1	0.1030	14.1	0.727	0.585	0.869	7.6	1.8	13.4	12.2	7.9	16.4
7	03-04-25	100	0.754	10.4	0.1103	14.6	0.729	0.588	0.871	7.7	1.8	13.6	12.3	8.0	16.7
8	04-08-25	100	0.746	7.8	0.0855	11.5	0.733	0.593	0.873	7.7	1.8	13.7	12.3	8.0	16.7
9	05-06-25	100	0.783	5.2	0.1099	14.0	0.732	0.593	0.871	7.8	2.0	13.6	12.5	8.1	16.8
10	06-03-25	100	0.920	4.4	0.1429	15.5	0.736	0.580	0.893	7.7	1.7	13.6	12.7	8.2	17.2
11	07-08-25	100	0.983	12.5	0.1150	11.7	0.754	0.570	0.938	7.9	1.5	14.2	12.7	8.1	17.2
12	08-05-25	100	0.959	2.7	0.0941	9.8	0.763	0.558	0.968	7.7	1.0	14.4	12.6	7.9	17.2
13	09-09-25	100	0.835	14.0	0.1126	13.5	0.767	0.559	0.975	7.8	0.9	14.7	12.3	8.4	16.2
14	10-07-25	100	0.774	5.0	0.0646	8.3	0.762	0.560	0.964	7.5	0.6	14.4	12.2	7.9	16.5
15	11-04-25	100	0.914	2.9	0.1313	14.4	0.775	0.569	0.981	7.3	0.1	14.4	12.2	7.8	16.6
16	12-02-25	100	0.629	5.0	0.0623	9.9	0.772	0.559	0.985	6.9	0.0	13.8	12.1	7.6	16.6
17	01-06-26	100	0.914	5.0	0.0736	8.1	0.783	0.564	1.002	6.8	-0.2	13.7	11.9	7.0	16.7
18	02-10-26	100	0.916	6.5	0.1204	13.1	0.795	0.568	1.022	6.8	-0.1	13.7	12.0	7.1	16.9
19	02-10-26	100	0.838	6.3	0.1170	14.0	0.797	0.575	1.019	6.8	0.0	13.5	12.1	7.3	16.9
20	03-03-26	100	0.822	8.6	0.1323	16.1	0.803	0.586	1.020	6.8	0.0	13.6	12.2	7.1	17.3

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

Reviewed and Authorized by Jim Sumerlin

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: **139**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2465				
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:	7 B
Spawn date:	03-24-26	*Artemia* CHM number:	CHM432
Hatch dates and times:	03-02-26 14:13 TO 03-03-26 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.20 S.U. Temperature = 24.6 °C	Date / Time in oven:	03-10-26 0620
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	03-11-26 0620
		*Final oven temperature:	60°C
		Total drying time:	24 HOURS

*60°C Oven, Thermometer SN: 14-98585

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	03-03-26	0505	JK	1350	JK	0705	JK	02-25-26 A
1	03-04-26	0500	JK	1100	JK	0700	JK	↓
2	03-05-26	0500	JK	1300	JK	0700	JK	02-25-26 B
3	03-06-26	0500	JK	1100	JK	0700	JK	↓
4	03-07-26	0655	JK	1250	JK	0855	JK	03-05-25 A
5	03-08-26	0640	JK	1200	JK	0840	JK	↓
6	03-09-26	0600	JK	1200	JK	0832	JK	
7	03-10-26					0620	JK	

Chemical analyses:

Parameter	Reporting Limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved Oxygen (D.O.)	1.0 mg/L	SM 4500-O H-2021	HACH HQ430d Flexi	SN250100050300
Conductivity	14.9 µmhos/cm	SM 2510 B-2021	Accumet AR20	93312452
Alkalinity	5.0 mg $CaCO_3$/L	SM 2320 B-2021	Accumet AR20	93312452
Hardness	5.0 mg $CaCO_3$/L	SM 2340 C-2021	Not applicable	Not applicable
Temperature	0.1°C	SM 2550B-2010	Digital Thermometer	130664685

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	07.	≤ 20%	7-day LC_{50} (mg/L KCl)	796.0
Average weight per initial larvae:	0.822		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.822	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	665.6

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **139**

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: Light pink Analyst: XL Date: 02-20-26	13.28	12.43	9.89	12.62	12.87	12.01	11.98	12.26	13.67	14.07	13.22	13.78
*B = Pan + Larvae weight (mg) Analyst: JL Date: 03-12-26	22.19	19.89	18.60	20.40	23.18	20.44	22.70	21.76	21.44	20.86	21.87	21.71
C = Larvae weight (mg) = B – A Analyst: JL	8.91	7.46	8.71	7.78	10.31	8.43	10.72	9.50	7.77	6.79	8.65	7.93
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.891	0.746	0.871	0.778	1.031	0.843	1.072	0.950	0.777	0.679	0.865	0.793
Average weight per initial number of larvae (mg)	0.822				0.974		-18.6%		0.779		5.2%	
Percent reduction from control (%)												

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **139**

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	9 1d	5 5d	5 5d	6 4d	5 5d
2	10	10	10	10	9	9	8 1d	9	5	4 1d	5 1d	5
3	10	10	10	10	9	9	9	9	4 B	4	5	4 B
4	10	10	10	10	8 1d	9	8	9	4	4	4 1d	3 1d
5	10	10	10	10	8	9	8	9	3 1d	3 1d	4	3
6	10	10	10	10	8	8 1d	8	9	2 1d	2 1d	2 2d	1 2d
7	10	10	9 1d	9 1d	8	8	7 1d	7 2d	2	1 1d	2	1
*A = Pan weight (mg) Tray color code: Light pink Analyst: XL Date: 02.20.26	13.33	13.41	12.78	13.04	13.53	13.75	12.58	12.72	13.33	13.02	13.97	13.04
*B = Pan + Larvae weight (mg) Analyst: JL Date: 03.12.26	21.69	21.94	19.99	19.75	19.82	19.45	17.77	18.13	14.97	13.80	15.70	14.08
C = Larvae weight (mg) = B – A Analyst: JL	8.36	8.53	7.21	6.71	6.29	5.70	5.19	5.41	1.64	0.78	1.73	1.04
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.836	0.853	0.721	0.671	0.629	0.570	0.519	0.541	0.164	0.078	0.173	0.104
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.758		7.8%		0.565		31.3%		0.130		84.2%	

*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: **139**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	2 Sd	2 Sd	2 Sd	2 Sd
2	1 d	2	2	1 d
3	0 1d	1 1d	2	1
4	0	1	2	1
5	0	1	2	1
6	0	1	1 d	0 1d
7	0	1	0 1d	0
*A = Pan weight (mg) Tray color code: Light pink Analyst: XL Date: 02.20.26	11.47	13.78	14.13	12.58
*B = Pan + Larvae weight (mg) Analyst: JH Date: 03.12.26		14.63		
C = Larvae weight (mg) = B – A Analyst: JH		0.85		
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0	0.085	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.021		97.4%	

*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.

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: 139

Test dates: March 03-10, 2026

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	13.28	22.19	8.91	0.891	0.822	8.6	0.891	100.0	0.822	8.6	Not applicable
	B	10	10	12.43	19.89	7.46	0.746			0.746				
	C	10	10	9.89	18.60	8.71	0.871			0.871				
	D	10	10	12.62	20.40	7.78	0.778			0.778				
300	E	10	10	12.87	23.18	10.31	1.031	0.974	10.4	1.031	100.0	0.974	10.4	-18.6
	F	10	10	12.01	20.44	8.43	0.843			0.843				
	G	10	10	11.98	22.70	10.72	1.072			1.072				
	H	10	10	12.26	21.76	9.50	0.950			0.950				
450	I	10	10	13.67	21.44	7.77	0.777	0.779	9.8	0.777	100.0	0.779	9.8	5.2
	J	10	10	14.07	20.86	6.79	0.679			0.679				
	K	10	10	13.22	21.87	8.65	0.865			0.865				
	L	10	10	13.78	21.71	7.93	0.793			0.793				
600	M	10	10	13.33	21.69	8.36	0.836	0.796	4.7	0.836	95.0	0.758	9.9	7.8
	N	10	10	13.91	21.94	8.03	0.803			0.803				
	O	10	9	12.78	19.99	7.21	0.801			0.721				
	P	10	9	13.04	19.75	6.71	0.746			0.671				
750	Q	10	8	13.53	19.82	6.29	0.786	0.753	4.4	0.629	75.0	0.565	8.4	31.3
	R	10	8	13.75	19.45	5.70	0.713			0.570				
	S	10	7	12.58	17.77	5.19	0.741			0.519				
	T	10	7	12.72	18.13	5.41	0.773			0.541				
900	U	10	2	13.33	14.97	1.64	0.820	0.876	13.1	0.164	15.0	0.130	35.6	84.2
	V	10	1	13.02	13.80	0.78	0.780			0.078				
	W	10	2	13.97	15.70	1.73	0.865			0.173				
	X	10	1	13.04	14.08	1.04	1.040			0.104				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.850	0.0	0.000	2.5	0.021	200.0	97.4
	Z	10	1	13.78	14.63	0.85	0.850			0.085				
	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: 0.1323 MSD = Minimum Significant Difference

PMSD: 16.1 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.

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by JH, Summer

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	3/3/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/10/2026	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	0.9000	0.9000
750	0.8000	0.8000	0.7000	0.7000
900	0.2000	0.1000	0.2000	0.1000
1050	0.0000	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	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.7500	0.7500	1.0492	0.9912	1.1071	6.383	4	10.00	10.00	10	40
*900	0.1500	0.1500	0.3927	0.3218	0.4636	20.862	4	10.00	10.00	34	40
*1050	0.0250	0.0250	0.1995	0.1588	0.3218	40.840	4	10.00	10.00	39	40

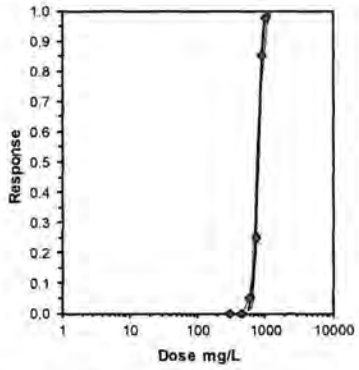
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91344	0.896	0.4009	-0.4477
Equality of variance cannot be confirmed				

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

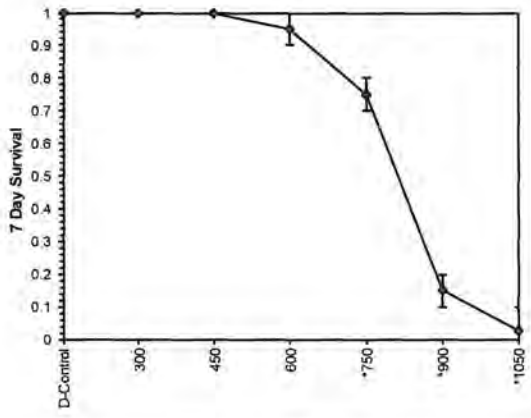
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	16.2208	2.08066	12.1427	20.2989	0	3.12278	9.48773	0.53749	2.90093	0.06165	3
Intercept	-42.056	6.05417	-53.922	-30.189							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	572.159	504.945	618.509
EC05	3.355	630.275	573.087	669.987
EC10	3.718	663.632	612.602	699.722
EC15	3.964	687.13	640.444	720.916
EC20	4.158	706.397	663.173	738.55
EC25	4.326	723.357	683.023	754.342
EC40	4.747	767.919	733.85	797.676
EC50	5.000	796.039	764.355	826.958
EC60	5.253	825.188	794.301	859.288
EC75	5.674	876.024	842.526	920.386
EC80	5.842	897.056	861.274	947.129
EC85	6.036	922.209	883.018	979.991
EC90	6.282	954.862	910.403	1023.81
EC95	6.645	1005.4	951.455	1093.66
EC99	7.326	1107.52	1031.33	1240.42



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by [initials]

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	3/3/2026	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/10/2026	Lab ID:	ETS-Envr. 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	0.8910	0.7460	0.8710	0.7780
300	1.0310	0.8430	1.0720	0.9500
450	0.7770	0.6790	0.8650	0.7930
600	0.8360	0.8030	0.7210	0.6710
750	0.6290	0.5700	0.5190	0.5410
900	0.1640	0.0780	0.1730	0.1040
1050	0.0000	0.0850	0.0000	0.0000

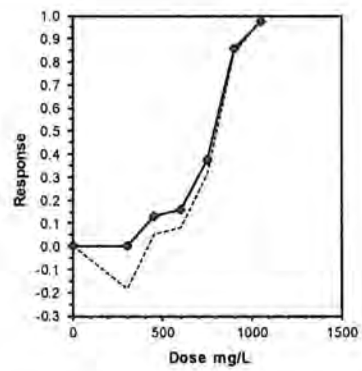
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.8215	1.0000	0.8215	0.7460	0.8910	8.571	4				0.8978	1.0000
300	0.9740	1.1856	0.9740	0.8430	1.0720	10.367	4	-2.640	2.290	0.1323	0.8978	1.0000
450	0.7785	0.9477	0.7785	0.6790	0.8650	9.837	4	0.744	2.290	0.1323	0.7785	0.8672
600	0.7578	0.9224	0.7578	0.6710	0.8360	9.948	4	1.103	2.290	0.1323	0.7578	0.8441
750	0.5648	0.6875	0.5648	0.5190	0.6290	8.438	4				0.5648	0.6291
900	0.1298	0.1579	0.1298	0.0780	0.1730	35.555	4				0.1298	0.1445
1050	0.0213	0.0259	0.0213	0.0000	0.0850	200.000	4				0.0213	0.0237

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93941	0.844	-0.3234	-1.2031
Bartlett's Test indicates equal variances (p = 0.93)	0.43297	11.3449		

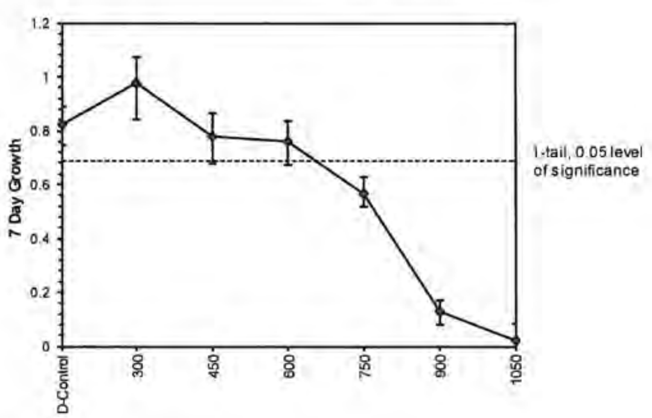
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.1323	0.16105	0.03819	0.00668	0.01144	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	356.46	30.24	324.93	455.78	3.8600
IC10	412.92	62.77	349.86	727.10	1.5814
IC15	561.42	80.51	319.57	682.22	0.1108
IC20	630.74	50.39	336.79	690.79	-1.8843
IC25	665.63	24.91	573.48	724.14	-0.7410
IC40	759.00	10.12	717.86	781.52	-0.9119
IC50	789.96	6.86	767.35	809.74	-0.0565



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **139**

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	[illegible]	XL	XL	XL	XL	XL
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.16	7.85	7.76	7.79	7.80	7.78
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	7.9	8.4	7.8
	Conductivity (µmhos/cm)	293		306		298	
	Alkalinity (mg $CaCO_3$/L)	63				62	
	Hardness (mg $CaCO_3$/L)	86			03-11-24	88	
	Temperature (°C)	24.7	25.1	24.6	24.9	24.9	24.9
300 mg KCl/L	pH (S.U.)	8.09	7.83	7.89	7.77	7.89	7.76
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	7.8	8.4	7.8
	Conductivity (µmhos/cm) 080326 741 → 1300A			814		810	
	Temperature (°C)	24.9	24.6	24.7	25.1	24.8	25.2
450 mg KCl/L	pH (S.U.)	8.09	7.83	7.90	7.78	7.90	7.77
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	7.8	8.4	7.8
	Conductivity (µmhos/cm) 080326 989 → 1054			1070		1060	
	Temperature (°C)	24.8	24.6	24.7	25.2	24.8	24.8
600 mg KCl/L	pH (S.U.)	8.09	7.84	7.91	7.78	7.91	7.78
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	7.8	8.4	7.8
	Conductivity (µmhos/cm)	1220		1300		1310	
	Temperature (°C)	24.6	25.0	24.7	24.6	24.8	24.6
750 mg KCl/L	pH (S.U.)	8.09	7.84	7.91	7.78	7.92	7.78
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	7.8	8.4	7.8
	Conductivity (µmhos/cm)	1440		1540		1520	
	Temperature (°C)	24.9	24.8	24.9	24.8	24.9	24.6
900 mg KCl/L	pH (S.U.)	8.09	7.85	7.91	7.78	7.92	7.80
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	7.7	8.4	7.8
	Conductivity (µmhos/cm)	1670		1790		1780	
	Temperature (°C)	24.9	25.0	24.9	24.6	24.9	25.0
1050 mg KCl/L	pH (S.U.)	8.09	7.85	7.92	7.80	7.93	7.81
	Dissolved oxygen (mg/L)	8.2	8.1	8.4	8.0	8.4	7.9
	Conductivity (µmhos/cm)	1910		2060		2040	
	Temperature (°C)	24.9	25.1	24.7	25.0	24.9	24.8
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **139**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	XL	BSL	BSL	BSL	BSL	XL	XL	MC
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.79	7.81	7.68	7.71	7.77	7.79	7.85	7.53
	Dissolved oxygen (mg/L)	8.4	7.9	8.6	7.3	8.5	7.8	8.4	6.1
	Conductivity (µmhos/cm)	302		~~278~~ ~~275~~ 290		281		298	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)		03-10-26 M	84					03-11-26 M
	Temperature (°C)	24.8	24.9	24.9	24.7	24.8	24.8	24.7	24.7
300 mg KCl/L	pH (S.U.)	7.92	7.79	7.90	7.71	7.99	7.76	7.97	7.50
	Dissolved oxygen (mg/L)	8.4	7.4	8.5	7.1	8.4	7.6	8.4	5.9
	Conductivity (µmhos/cm)	812		756		779		810	
	Temperature (°C)	24.9	25.0	24.8	25.1	24.9	25.0	24.7	24.6
450 mg KCl/L	pH (S.U.)	7.92	7.79	7.90	7.71	8.00	7.76	7.98	7.50
	Dissolved oxygen (mg/L)	8.4	7.7	8.6	7.1	8.5	7.6	8.4	5.1
	Conductivity (µmhos/cm)	1050		992		1010		1030	
	Temperature (°C)	25.0	25.0	24.8	24.8	24.7	25.0	24.8	24.6
600 mg KCl/L	pH (S.U.)	7.94	7.78	7.91	7.72	8.01	7.75	7.98	7.50
	Dissolved oxygen (mg/L)	8.4	7.3	8.6	7.2	8.5	7.6	8.4	5.7
	Conductivity (µmhos/cm)	1300		1220		1240		1280	
	Temperature (°C)	25.0	25.0	25.0	24.8	24.7	24.9	24.7	24.6
750 mg KCl/L	pH (S.U.)	7.95	7.77	7.92	7.72	8.01	7.75	7.99	7.50
	Dissolved oxygen (mg/L)	8.4	7.4	8.6	7.2	8.5	7.7	8.4	5.8
	Conductivity (µmhos/cm)	1550		1450		1490		1540	
	Temperature (°C)	25.0	24.7	25.0	24.9	24.7	24.9	24.7	24.6
900 mg KCl/L	pH (S.U.)	7.95	7.78	7.93	7.73	8.03	7.76	7.99	7.51
	Dissolved oxygen (mg/L)	8.4	7.7	8.5	7.4	8.5	7.7	8.4	6.7
	Conductivity (µmhos/cm)	1790		1690		1730		1750	
	Temperature (°C)	25.0	24.7	24.9	25.0	24.8	24.9	24.9	24.5
1050 mg KCl/L	pH (S.U.)	7.96	7.79	7.93	7.76	8.02	7.76	7.99	7.52
	Dissolved oxygen (mg/L)	8.4	7.9	8.5	7.7	8.5	7.5	8.4	5.4
	Conductivity (µmhos/cm)	2060		1920		1980		2030	
	Temperature (°C)	25.0	24.7	24.9	24.8	24.8	25.1	24.9	24.8
		Initial	Final	Initial	Final	Initial	Final	Initial	Final