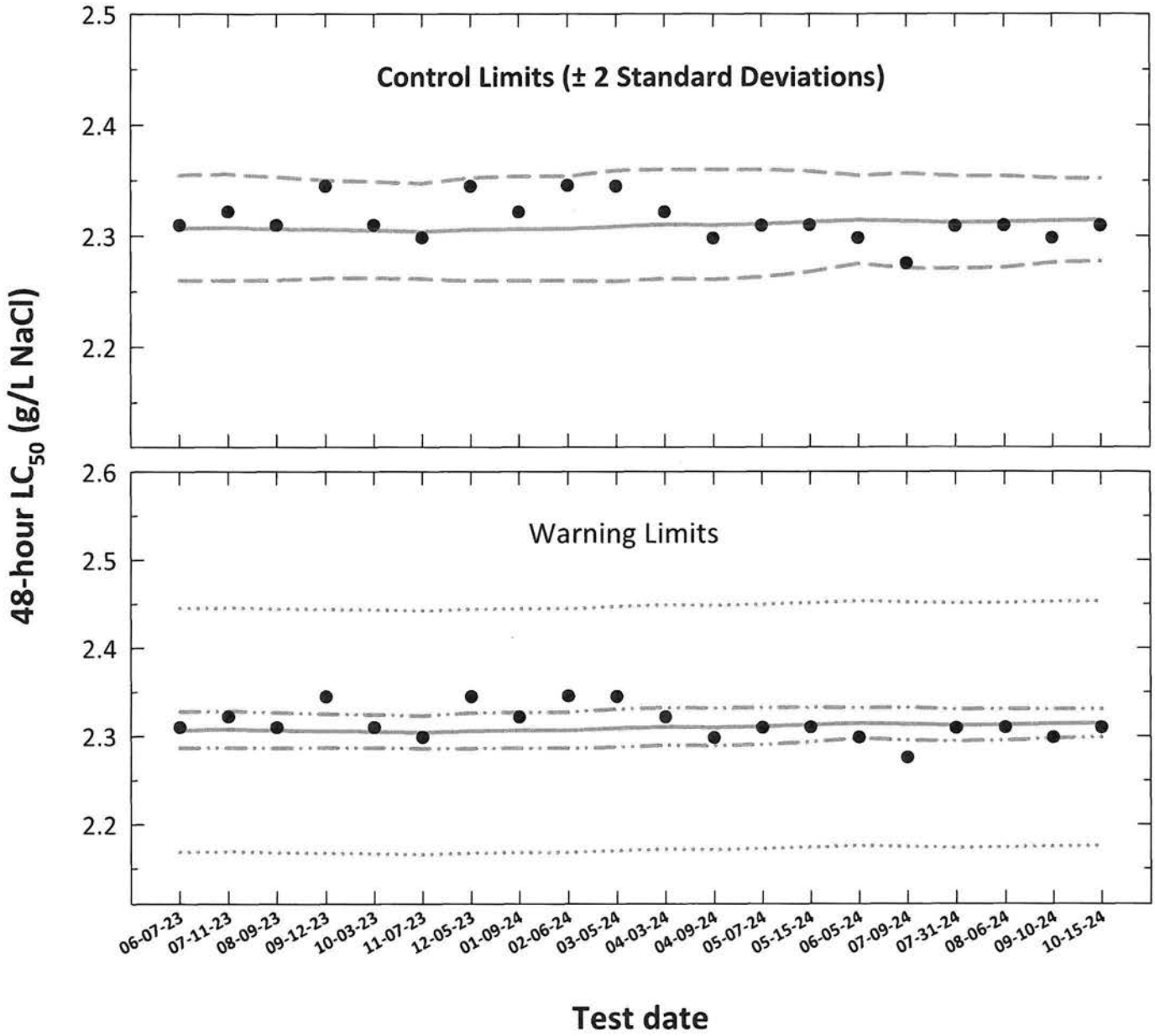
Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	06-07-23	2.3099	0.3636	0.3630	0.0045	2.3070	2.2600	2.3549	2.2866	2.3278	2.1686	2.4454
2	07-11-23	2.3220	0.3659	0.3632	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286	2.1691	2.4460
3	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268	2.1681	2.4448
4	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251	2.1675	2.4442
5	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670	2.4436
6	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229	2.1659	2.4424
7	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263	2.1675	2.4442
8	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271	2.1681	2.4449
9	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272	2.1681	2.4449
10	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307	2.1703	2.4474
11	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321	2.1719	2.4492
12	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317	2.1714	2.4486
13	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323	2.1725	2.4498
14	05-15-24	2.3099	0.3636	0.3642	0.0043	2.3129	2.2680	2.3586	2.2935	2.3327	2.1741	2.4516
15	06-05-24	2.2984	0.3614	0.3645	0.0037	2.3146	2.2753	2.3546	2.2976	2.3319	2.1757	2.4535
16	07-09-24	2.2755	0.3571	0.3643	0.0040	2.3135	2.2710	2.3567	2.2951	2.3322	2.1747	2.4523
17	07-31-24	2.3091	0.3634	0.3640	0.0039	2.3122	2.2708	2.3544	2.2943	2.3304	2.1735	2.4510
18	08-06-24	2.3096	0.3635	0.3641	0.0039	2.3128	2.2719	2.3544	2.2951	2.3308	2.1740	2.4516
19	09-10-24	2.2984	0.3614	0.3644	0.0036	2.3140	2.2763	2.3522	2.2977	2.3305	2.1751	2.4528
20	10-15-24	2.3096	0.3635	0.3645	0.0035	2.3145	2.2775	2.3522	2.2985	2.3308	2.1756	2.4534

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC_{50} values.

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

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

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Summer

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 # 416

Dilution Preparation:

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

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

Stock solution INSS #: 2320

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	K	K	Bs
Control, MHSW	pH (S.U.)	7.75	7.68	7.38
	Dissolved oxygen (mg/L)	7.9	7.8	7.8
	Conductivity (µmhos/cm)	310		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	25.0	25.3	25.1
1750 mg/L	pH (S.U.)	7.90	7.64	7.53
	Dissolved oxygen (mg/L)	7.9	7.7	7.7
	Conductivity (µmhos/cm)	3500		
	Temperature (°C)	24.9	25.0	24.9
2000 mg/L	pH (S.U.)	7.89	7.65	7.57
	Dissolved oxygen (mg/L)	7.0	7.8	7.7
	Conductivity (µmhos/cm)	3920		
	Temperature (°C)	24.9	25.2	24.9
2250 mg/L	pH (S.U.)	7.89	7.65	7.59
	Dissolved oxygen (mg/L)	7.0	7.8	7.8
	Conductivity (µmhos/cm)	4320		
	Temperature (°C)	25.0	25.2	25.3
2500 mg/L	pH (S.U.)	7.88	7.65	7.60
	Dissolved oxygen (mg/L)	7.0	7.8	7.8
	Conductivity (µmhos/cm)	4730		
	Temperature (°C)	25.0	25.0	25.0
2750 mg/L	pH (S.U.)	7.89	7.66	7.60
	Dissolved oxygen (mg/L)	7.0	7.8	7.9
	Conductivity (µmhos/cm)	5200		
	Temperature (°C)	25.0	25.0	24.9

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 numbe
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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 applicabl
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066 46

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 # 416

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	10-15-24	*0500	JL	0700	JL	2B2	RED	10-13-24A
24	10-16-24			0650	JL			
48 Termination	10-17-24			0658	JL			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	10-08-24 D
Age:	< 24-hours old
Date and time organisms were born between:	10-14-24 1710 TO 10-15-24 0500
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.81
	Temperature (°C): 24.9

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

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

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

Comment codes: d = dead, u = unhealthy

Statistics:

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

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	10/15/2024	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	10/17/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

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

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

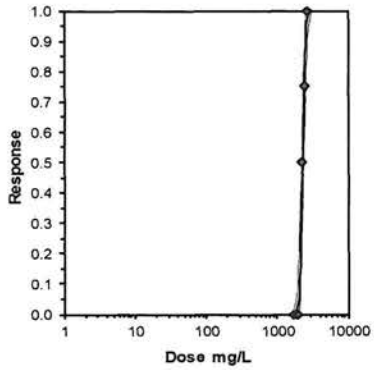
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77068	0.868	0.14468	4.85271
Equality of variance cannot be confirmed				

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

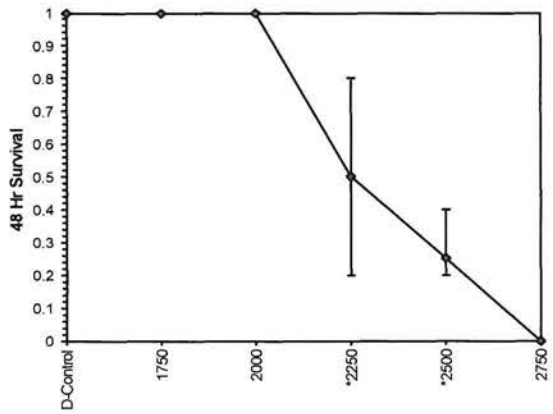
Maximum Likelihood-Probit

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

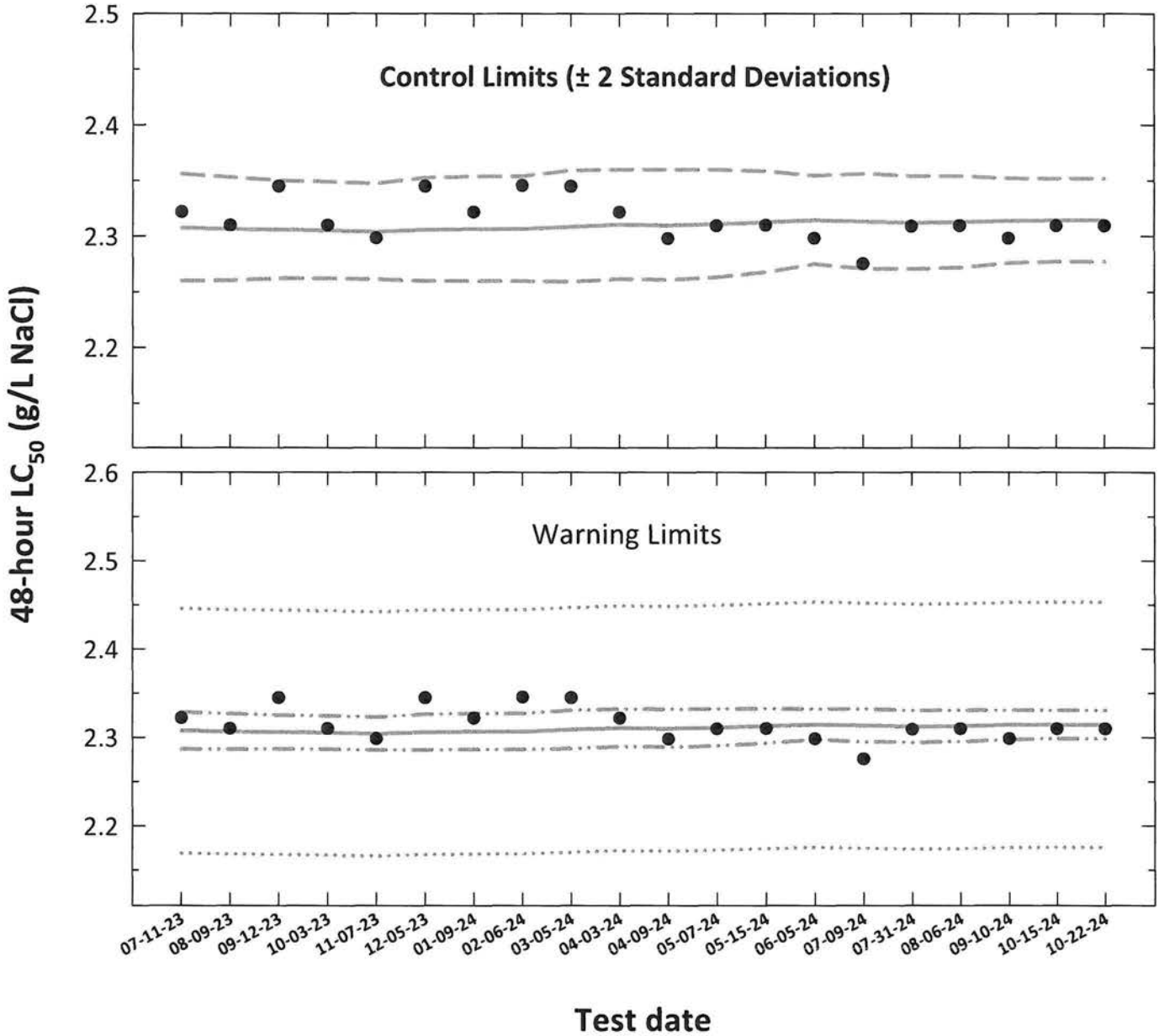
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1918.7	1718.88	2029.87
EC05	3.355	2025.8	1864.5	2117.99
EC10	3.718	2085.31	1945.73	2168.05
EC15	3.964	2126.45	2001.56	2203.56
EC20	4.158	2159.72	2046.25	2233.09
EC25	4.326	2188.68	2084.59	2259.6
EC40	4.747	2263.39	2179.46	2333.09
EC50	5.000	2309.55	2233.77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



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	07-11-23	2.3220	0.3659	0.3632	0.0045	2.3076	2.2601	2.3560	2.2870	2.3286	2.1691	2.4460
2	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268	2.1681	2.4448
3	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251	2.1675	2.4442
4	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670	2.4436
5	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229	2.1659	2.4424
6	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263	2.1675	2.4442
7	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271	2.1681	2.4449
8	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272	2.1681	2.4449
9	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307	2.1703	2.4474
10	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321	2.1719	2.4492
11	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317	2.1714	2.4486
12	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323	2.1725	2.4498
13	05-15-24	2.3099	0.3636	0.3642	0.0043	2.3129	2.2680	2.3586	2.2935	2.3327	2.1741	2.4516
14	06-05-24	2.2984	0.3614	0.3645	0.0037	2.3146	2.2753	2.3546	2.2976	2.3319	2.1757	2.4535
15	07-09-24	2.2755	0.3571	0.3643	0.0040	2.3135	2.2710	2.3567	2.2951	2.3322	2.1747	2.4523
16	07-31-24	2.3091	0.3634	0.3640	0.0039	2.3122	2.2708	2.3544	2.2943	2.3304	2.1735	2.4510
17	08-06-24	2.3096	0.3635	0.3641	0.0039	2.3128	2.2719	2.3544	2.2951	2.3308	2.1740	2.4516
18	09-10-24	2.2984	0.3614	0.3644	0.0036	2.3140	2.2763	2.3522	2.2977	2.3305	2.1751	2.4528
19	10-15-24	2.3096	0.3635	0.3645	0.0035	2.3145	2.2775	2.3522	2.2985	2.3308	2.1756	2.4534
20	10-22-24	2.3096	0.3635	0.3645	0.0035	2.3145	2.2774	2.3522	2.2985	2.3308	2.1756	2.4534

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC_{50} values.

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

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

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Summer

ETS

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 # 417

Dilution Preparation:

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

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

Stock solution INSS #: 2320

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	JC	BM	BM
Control, MHSW	pH (S.U.)	7.94	7.52	7.47
	Dissolved oxygen (mg/L)	7.8	7.7	7.8
	Conductivity (µmhos/cm)	307		
	Alkalinity (mg/L $CaCO_3$)	58		
	Hardness (mg/L $CaCO_3$)	84		
	Temperature (°C)	24.9	25.2	25.0
1750 mg/L	pH (S.U.)	7.94	7.56	7.62
	Dissolved oxygen (mg/L)	7.8	7.8	7.7
	Conductivity (µmhos/cm)	3360		
	Temperature (°C)	24.9	24.8	25.2
2000 mg/L	pH (S.U.)	7.92	7.63	7.63
	Dissolved oxygen (mg/L)	7.7	7.9	7.7
	Conductivity (µmhos/cm)	3880		
	Temperature (°C)	24.9	24.9	25.2
2250 mg/L	pH (S.U.)	7.92	7.64	7.64
	Dissolved oxygen (mg/L)	7.7	7.9	7.7
	Conductivity (µmhos/cm)	4260		
	Temperature (°C)	25.0	25.1	25.1
2500 mg/L	pH (S.U.)	7.91	7.66	7.64
	Dissolved oxygen (mg/L)	7.7	7.8	7.9
	Conductivity (µmhos/cm)	4800		
	Temperature (°C)	25.0	24.8	24.8
2750 mg/L	pH (S.U.)	7.91	7.66	7.69
	Dissolved oxygen (mg/L)	7.7	7.9	7.9
	Conductivity (µmhos/cm)	5140		
	Temperature (°C)	25.0	25.0	25.1

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measureme 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 G-2021	YSI Model S2CE	08A100271
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	13066461

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 # 417

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	10-22-24	* 0800	M	1000	M	2B1	RED	10-20-24
24	10-23-24			1000	M			
48 Termination	10-24-24			1000	M			

*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):	10-15-24 A
Age:	< 24-hours old
Date and time organisms were born between:	10-21-24 1528 TO 10-22-24 0800
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.83
	Temperature (°C): 25.0°C

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

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	4 u	5	5	4 u	3 u	5	3 u	4 u
48 Termination	2 d	4 u	2 d	2 d	1 d	3 d	0 d	1 d	0 d	0 d	0 d	0 d
Mean Survival	50%				75%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2233.6
Upper 95% confidence limit (mg NaCl/L)	2383.6
48-hour LC_{50} (mg NaCl/L)	2309.6

Comments:

Test Reviewed by: M

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	10/22/2024	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	10/24/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

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

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5000	0.5000	0.7903	0.6847	1.1071	26.725	4	10.00	10.00	10	20
*2500	0.2500	0.2500	0.5097	0.2255	0.8861	53.926	4	10.00	10.00	15	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

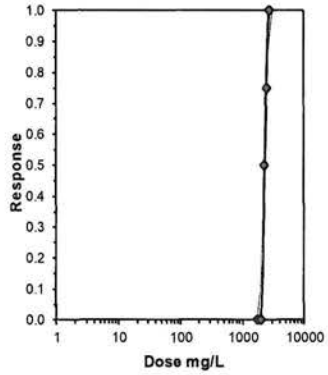
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.7254	0.868	1.30729	3.89446
Equality of variance cannot be confirmed				

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

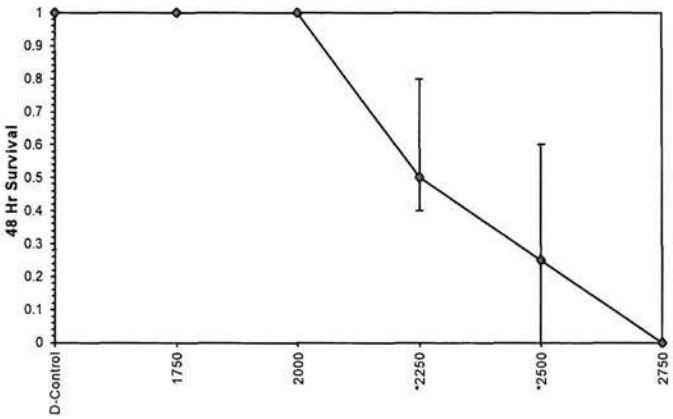
Maximum Likelihood-Probit

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

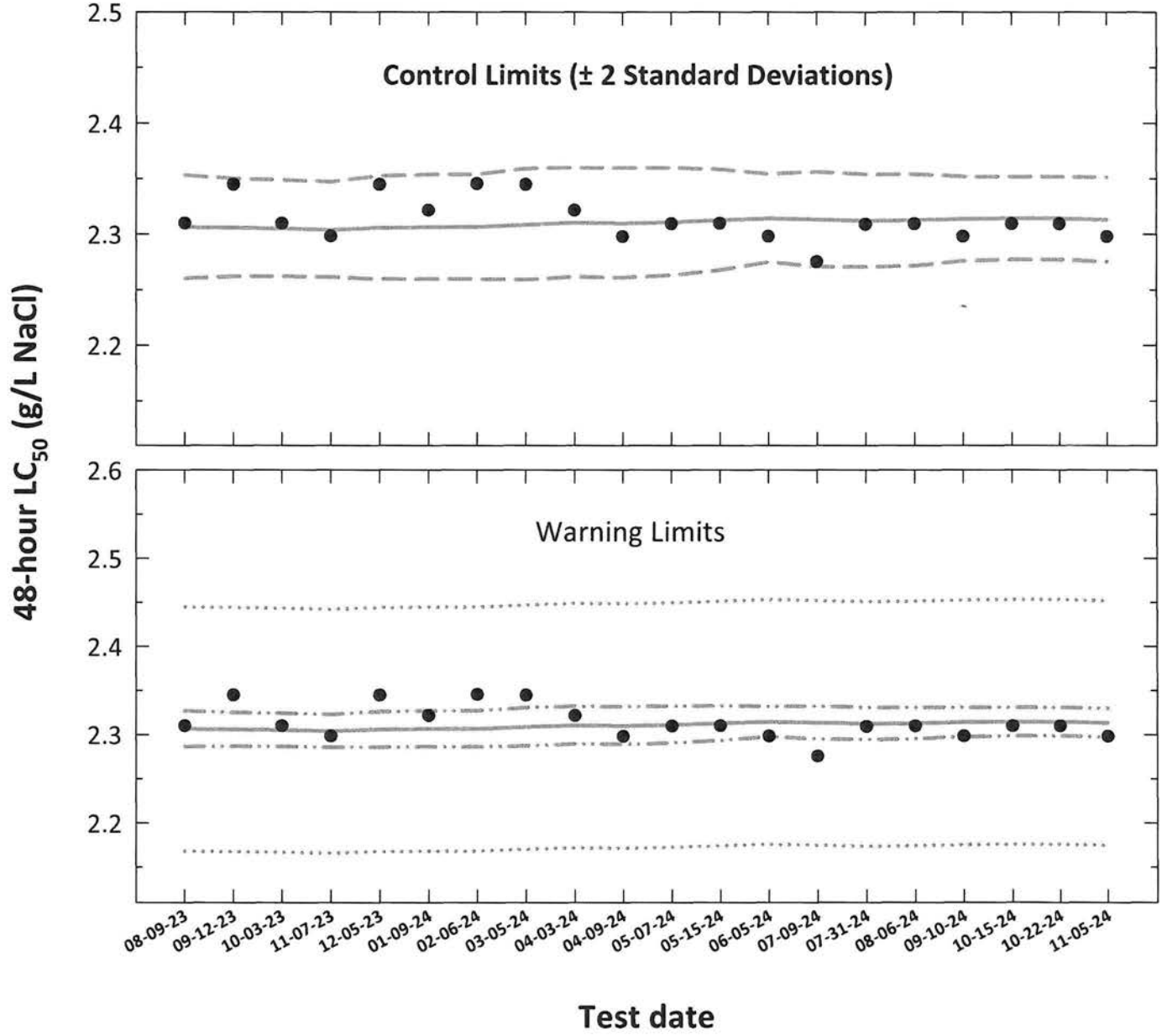
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1918.7	1718.88	2029.87
EC05	3.355	2025.8	1864.5	2117.99
EC10	3.718	2085.31	1945.73	2168.05
EC15	3.964	2126.45	2001.56	2203.56
EC20	4.158	2159.72	2046.25	2233.09
EC25	4.326	2188.68	2084.59	2259.6
EC40	4.747	2263.39	2179.46	2333.09
EC50	5.000	2309.55	2233.77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



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	08-09-23	2.3099	0.3636	0.3629	0.0044	2.3064	2.2605	2.3534	2.2865	2.3268	2.1681	2.4448
2	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251	2.1675	2.4442
3	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670	2.4436
4	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229	2.1659	2.4424
5	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263	2.1675	2.4442
6	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271	2.1681	2.4449
7	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272	2.1681	2.4449
8	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307	2.1703	2.4474
9	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321	2.1719	2.4492
10	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317	2.1714	2.4486
11	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323	2.1725	2.4498
12	05-15-24	2.3099	0.3636	0.3642	0.0043	2.3129	2.2680	2.3586	2.2935	2.3327	2.1741	2.4516
13	06-05-24	2.2984	0.3614	0.3645	0.0037	2.3146	2.2753	2.3546	2.2976	2.3319	2.1757	2.4535
14	07-09-24	2.2755	0.3571	0.3643	0.0040	2.3135	2.2710	2.3567	2.2951	2.3322	2.1747	2.4523
15	07-31-24	2.3091	0.3634	0.3640	0.0039	2.3122	2.2708	2.3544	2.2943	2.3304	2.1735	2.4510
16	08-06-24	2.3096	0.3635	0.3641	0.0039	2.3128	2.2719	2.3544	2.2951	2.3308	2.1740	2.4516
17	09-10-24	2.2984	0.3614	0.3644	0.0036	2.3140	2.2763	2.3522	2.2977	2.3305	2.1751	2.4528
18	10-15-24	2.3096	0.3635	0.3645	0.0035	2.3145	2.2775	2.3522	2.2985	2.3308	2.1756	2.4534
19	10-22-24	2.3096	0.3635	0.3645	0.0035	2.3145	2.2774	2.3522	2.2985	2.3308	2.1756	2.4534
20	11-05-24	2.2980	0.3613	0.3642	0.0036	2.3133	2.2757	2.3515	2.2970	2.3298	2.1745	2.4521

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC_{50} values.

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

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

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

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

Ceriodaphnia dubia Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 418

Dilution Preparation:

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

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

Stock solution INSS #: 2331

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	[illegible]	[illegible]	[illegible]
Control, MHSW	pH (S.U.)	7.71	7.49	7.41
	Dissolved oxygen (mg/L)	7.7	7.7	7.7
	Conductivity (µmhos/cm)	298		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	92		
	Temperature (°C)	25.0	25.2	25.0
1750 mg/L	pH (S.U.)	7.93	7.55	7.55
	Dissolved oxygen (mg/L)	7.9	7.7	7.6
	Conductivity (µmhos/cm)	3390		
	Temperature (°C)	25.2	25.1	25.2
2000 mg/L	pH (S.U.)	7.93	7.59	7.56
	Dissolved oxygen (mg/L)	7.9	7.6	7.9
	Conductivity (µmhos/cm)	3850		
	Temperature (°C)	25.2	25.1	25.2
2250 mg/L	pH (S.U.)	7.93	7.62	7.58
	Dissolved oxygen (mg/L)	7.8	7.7	7.9
	Conductivity (µmhos/cm)	4320		
	Temperature (°C)	25.2	25.3	24.9
2500 mg/L	pH (S.U.)	7.93	7.63	7.60
	Dissolved oxygen (mg/L)	7.8	7.7	7.9
	Conductivity (µmhos/cm)	4640		
	Temperature (°C)	25.1	25.0	24.9
2750 mg/L	pH (S.U.)	7.92	7.63	7.62
	Dissolved oxygen (mg/L)	7.9	7.8	7.9
	Conductivity (µmhos/cm)	5210		
	Temperature (°C)	25.1	25.2	25.1

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial number
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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	130646PS

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 # 418

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	11-05-24	* 0415	JL	0615	JL	2 B4	RCD	10-30-24A
24	11-06-24			0605	JL			
48 Termination	11-07-24			0606	JL			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	10-29-24 D
Age:	< 24-hours old
Date and time organisms were born between:	11-04-24 1458 TO 11-05-24 0415
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.74
	Temperature (°C): 25.2

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

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	4 1d	5	5	4 1d	2 3d	3 2d	3 2d	3 2d
48 Termination	2 3d	2 3d	2 3d	3 2d	1 3d	1 4d	1 4d	2 2d	0 2d	0 3d	0 3d	0 3d
Mean Survival	45%				25%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2221.6
Upper 95% confidence limit (mg NaCl/L)	2372.6
48-hour LC_{50} (mg NaCl/L)	2298.0

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	11/5/2024	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	11/7/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

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

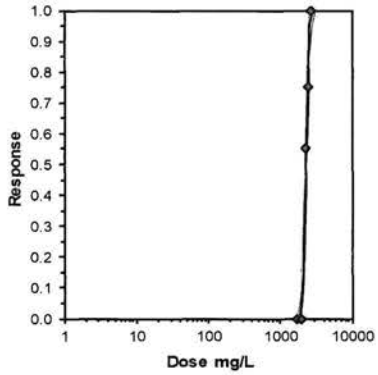
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.4500	0.4500	0.7351	0.6847	0.8861	13.697	4	10.00	10.00	11	20
*2500	0.2500	0.2500	0.5189	0.4636	0.6847	21.301	4	10.00	10.00	15	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.66241	0.868	1.98367	4.13297
Equality of variance cannot be confirmed				

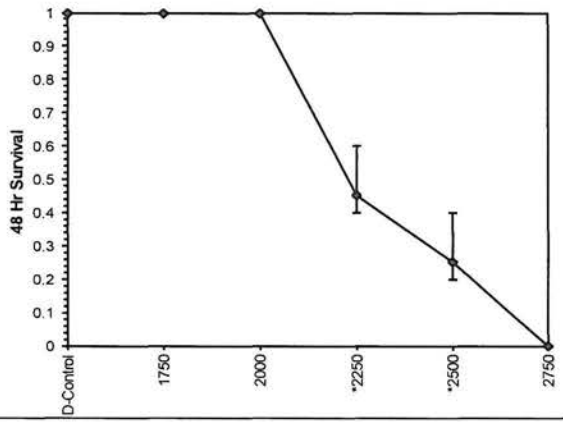
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2000	2250	2121.32	
Treatments vs D-Control				

					Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	28.2327	5.00204	18.4287	38.0367	0	4.68438	7.81472	0.19642	3.36135	0.03542	7
Intercept	-89.9	16.8293	-122.89	-56.914							
TSCR											

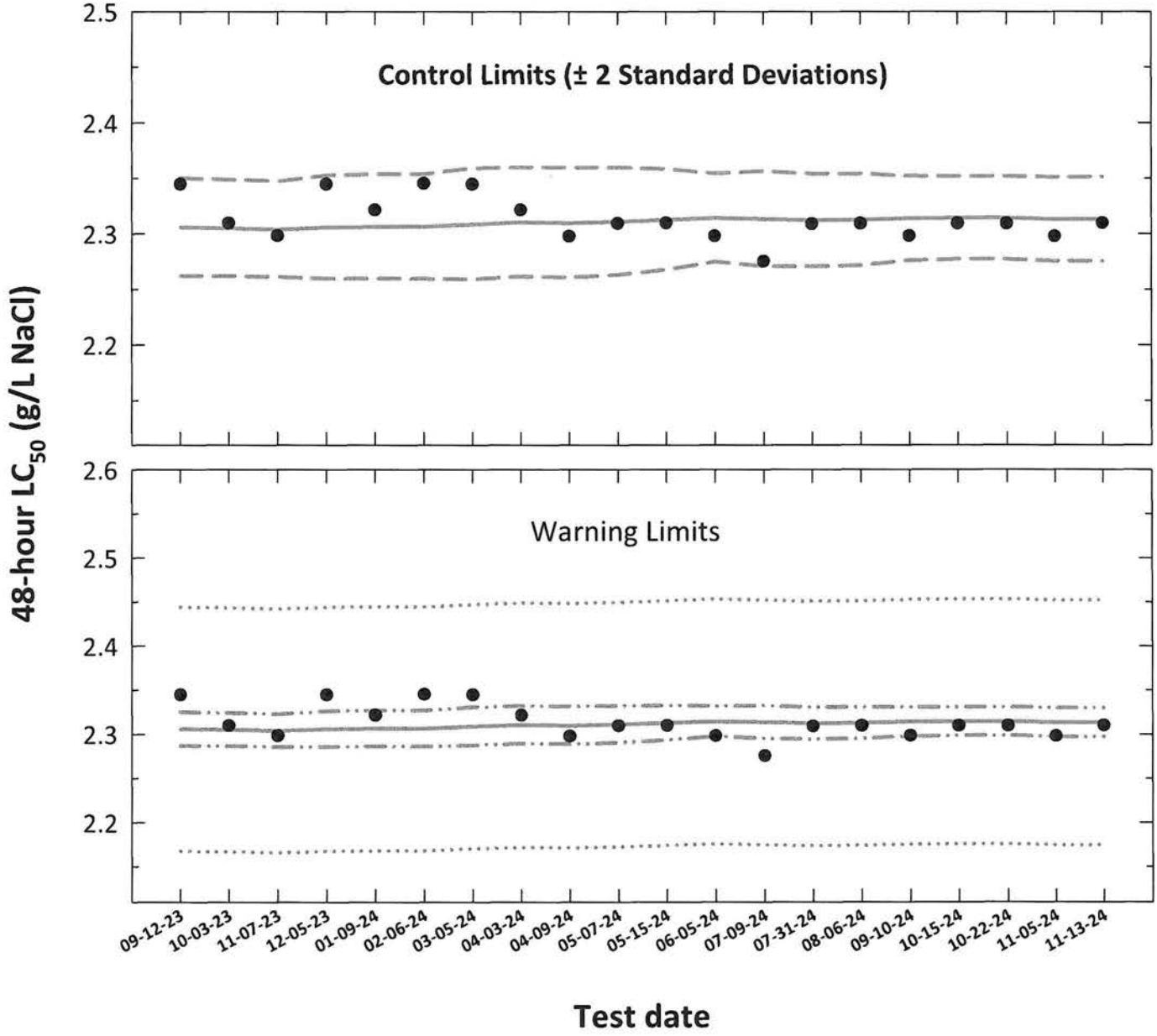
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1900.85	1700.87	2012.91
EC05	3.355	2009.49	1847.79	2102.51
EC10	3.718	2069.92	1929.85	2153.44
EC15	3.964	2111.72	1986.3	2189.56
EC20	4.158	2145.54	2031.51	2219.6
EC25	4.326	2174.99	2070.32	2246.57
EC40	4.747	2250.99	2166.47	2321.29
EC50	5.000	2297.98	2221.6	2372.6
EC60	5.253	2345.96	2273.62	2429.86
EC75	5.674	2427.94	2353.13	2538.5
EC80	5.842	2461.26	2382.8	2585.82
EC85	6.036	2500.67	2416.48	2643.6
EC90	6.282	2551.17	2457.92	2719.92
EC95	6.645	2627.89	2518.36	2839.69
EC99	7.326	2778.09	2631.39	3083.9



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	09-12-23	2.3449	0.3701	0.3628	0.0041	2.3059	2.2623	2.3503	2.2870	2.3251	2.1675	2.4442
2	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670	2.4436
3	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229	2.1659	2.4424
4	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263	2.1675	2.4442
5	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271	2.1681	2.4449
6	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272	2.1681	2.4449
7	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307	2.1703	2.4474
8	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321	2.1719	2.4492
9	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317	2.1714	2.4486
10	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323	2.1725	2.4498
11	05-15-24	2.3099	0.3636	0.3642	0.0043	2.3129	2.2680	2.3586	2.2935	2.3327	2.1741	2.4516
12	06-05-24	2.2984	0.3614	0.3645	0.0037	2.3146	2.2753	2.3546	2.2976	2.3319	2.1757	2.4535
13	07-09-24	2.2755	0.3571	0.3643	0.0040	2.3135	2.2710	2.3567	2.2951	2.3322	2.1747	2.4523
14	07-31-24	2.3091	0.3634	0.3640	0.0039	2.3122	2.2708	2.3544	2.2943	2.3304	2.1735	2.4510
15	08-06-24	2.3096	0.3635	0.3641	0.0039	2.3128	2.2719	2.3544	2.2951	2.3308	2.1740	2.4516
16	09-10-24	2.2984	0.3614	0.3644	0.0036	2.3140	2.2763	2.3522	2.2977	2.3305	2.1751	2.4528
17	10-15-24	2.3096	0.3635	0.3645	0.0035	2.3145	2.2775	2.3522	2.2985	2.3308	2.1756	2.4534
18	10-22-24	2.3096	0.3635	0.3645	0.0035	2.3145	2.2774	2.3522	2.2985	2.3308	2.1756	2.4534
19	11-05-24	2.2980	0.3613	0.3642	0.0036	2.3133	2.2757	2.3515	2.2970	2.3298	2.1745	2.4521
20	11-13-24	2.3099	0.3636	0.3642	0.0036	2.3133	2.2757	2.3515	2.2970	2.3298	2.1745	2.4521

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC_{50} values.

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

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

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Prepared 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 # 419

Dilution Preparation:

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

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

Stock solution INSS #: 2331

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	[illegible]	[illegible]	[illegible]
Control, MHSW	pH (S.U.)	7.66	7.52	7.69
	Dissolved oxygen (mg/L)	7.9	7.6	7.7
	Conductivity (µmhos/cm)	302		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	90		
	Temperature (°C)	25.2	25.0	25.3
1750 mg/L	pH (S.U.)	7.89	7.70	7.70
	Dissolved oxygen (mg/L)	7.9	7.6	7.7
	Conductivity (µmhos/cm)	3410		
	Temperature (°C)	25.0	25.2	25.1
2000 mg/L	pH (S.U.)	7.86	7.71	7.69
	Dissolved oxygen (mg/L)	7.8	7.6	7.7
	Conductivity (µmhos/cm)	3830		
	Temperature (°C)	25.1	25.2	25.1
2250 mg/L	pH (S.U.)	7.84	7.75	7.74
	Dissolved oxygen (mg/L)	7.8	7.7	7.7
	Conductivity (µmhos/cm)	4210		
	Temperature (°C)	25.1	25.0	24.9
2500 mg/L	pH (S.U.)	7.82	7.75	7.73
	Dissolved oxygen (mg/L)	7.8	7.7	7.8
	Conductivity (µmhos/cm)	4720		
	Temperature (°C)	25.1	25.2	24.9
2750 mg/L	pH (S.U.)	7.82	7.79	7.75
	Dissolved oxygen (mg/L)	7.8	7.7	7.8
	Conductivity (µmhos/cm)	5190		
	Temperature (°C)	24.9	25.2	24.9

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 G-2021	YSI Model 52CE	08A100271
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	13066465

ETS

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 # 419

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	11-13-24	* 0500	M	0700	M	2B1	RED	11-08-24
24	11-14-24			0700	M			
48 Termination	11-15-24			0704	M			

*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):	11-06-24 D
Age:	< 24-hours old
Date and time organisms were born between:	11-12-24 1453 TO 11-13-24 0500
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.82
	Temperature (°C): 25.0 - C

Survival Data (number of living organisms):

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

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

Comment codes: d = dead, u = unhealthy

Statistics:

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

Comments:

Test Reviewed by: M

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	11/13/2024	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	11/15/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

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

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5500	0.5500	0.8407	0.6847	1.1071	23.960	4	10.00	10.00	9	20
*2500	0.2000	0.2000	0.4594	0.2255	0.6847	40.823	4	10.00	10.00	16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

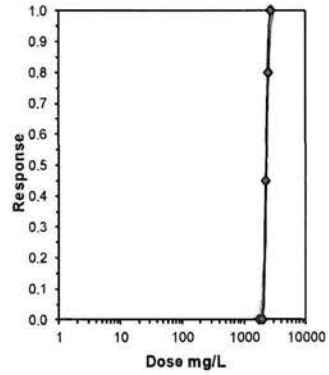
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.74905	0.868	0.45087	2.42089
Equality of variance cannot be confirmed				

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

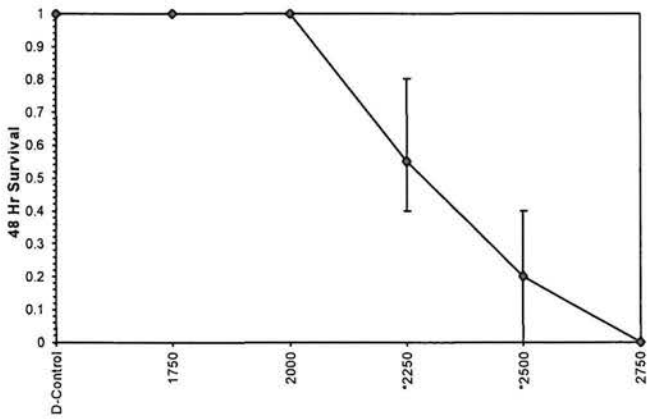
Maximum Likelihood-Probit

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

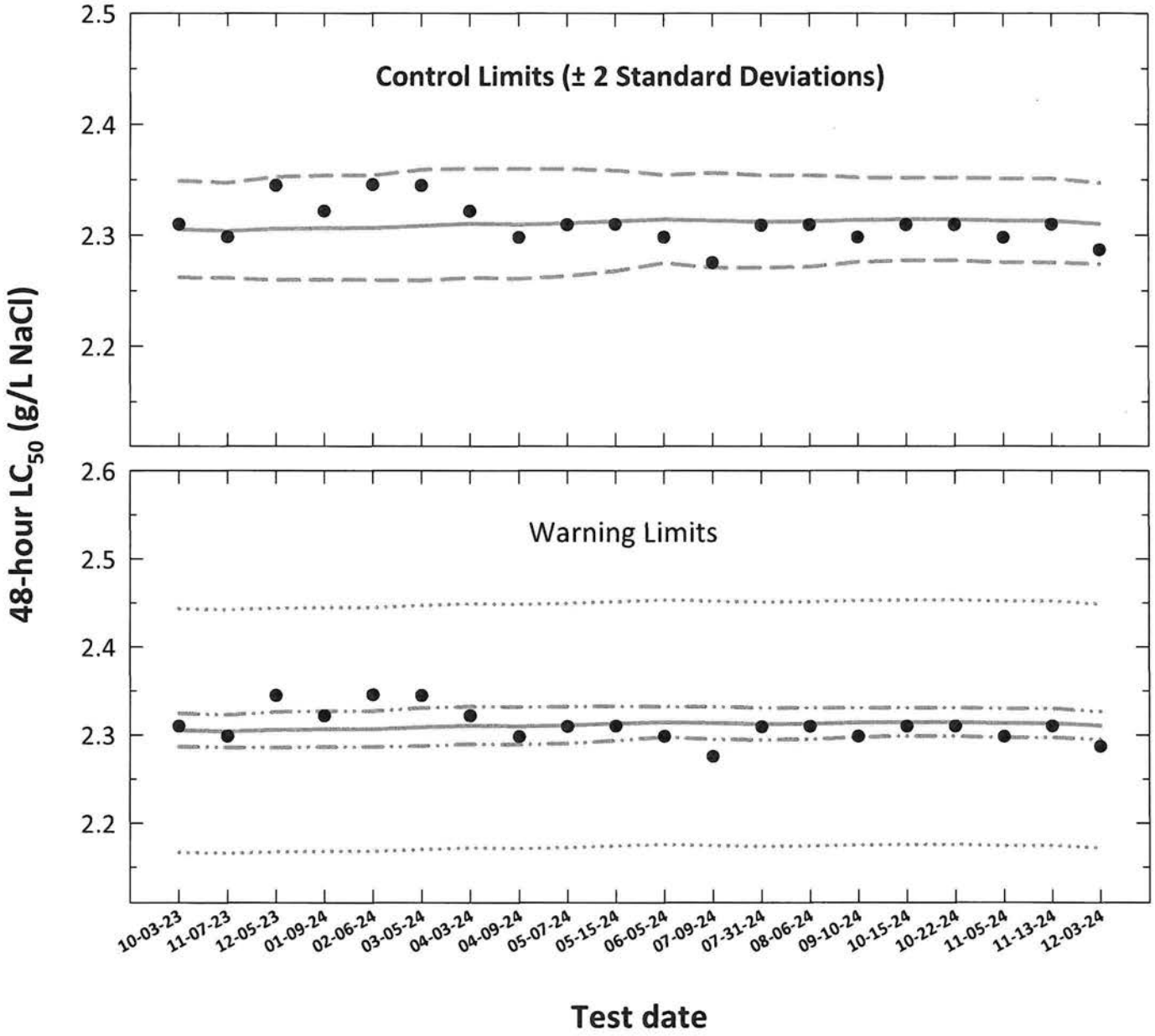
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1948.47	1752	2054.85
EC05	3.355	2048.07	1890.23	2136.13
EC10	3.718	2103.23	1966.98	2182.25
EC15	3.964	2141.28	2019.59	2214.95
EC20	4.158	2172.02	2061.61	2242.15
EC25	4.326	2198.74	2097.59	2266.57
EC40	4.747	2267.53	2186.32	2334.36
EC50	5.000	2309.94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



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	10-03-23	2.3099	0.3636	0.3627	0.0041	2.3053	2.2622	2.3492	2.2866	2.3243	2.1670	2.4436
2	11-07-23	2.2985	0.3614	0.3625	0.0040	2.3042	2.2617	2.3474	2.2857	2.3229	2.1659	2.4424
3	12-05-23	2.3449	0.3701	0.3628	0.0044	2.3059	2.2598	2.3529	2.2859	2.3263	2.1675	2.4442
4	01-09-24	2.3217	0.3658	0.3630	0.0044	2.3065	2.2599	2.3540	2.2863	2.3271	2.1681	2.4449
5	02-06-24	2.3455	0.3702	0.3630	0.0044	2.3065	2.2598	2.3542	2.2863	2.3272	2.1681	2.4449
6	03-05-24	2.3449	0.3701	0.3634	0.0047	2.3088	2.2594	2.3593	2.2874	2.3307	2.1703	2.4474
7	04-03-24	2.3217	0.3658	0.3637	0.0046	2.3106	2.2619	2.3603	2.2895	2.3321	2.1719	2.4492
8	04-09-24	2.2980	0.3613	0.3636	0.0047	2.3100	2.2610	2.3600	2.2888	2.3317	2.1714	2.4486
9	05-07-24	2.3096	0.3635	0.3638	0.0045	2.3111	2.2633	2.3600	2.2905	2.3323	2.1725	2.4498
10	05-15-24	2.3099	0.3636	0.3642	0.0043	2.3129	2.2680	2.3586	2.2935	2.3327	2.1741	2.4516
11	06-05-24	2.2984	0.3614	0.3645	0.0037	2.3146	2.2753	2.3546	2.2976	2.3319	2.1757	2.4535
12	07-09-24	2.2755	0.3571	0.3643	0.0040	2.3135	2.2710	2.3567	2.2951	2.3322	2.1747	2.4523
13	07-31-24	2.3091	0.3634	0.3640	0.0039	2.3122	2.2708	2.3544	2.2943	2.3304	2.1735	2.4510
14	08-06-24	2.3096	0.3635	0.3641	0.0039	2.3128	2.2719	2.3544	2.2951	2.3308	2.1740	2.4516
15	09-10-24	2.2984	0.3614	0.3644	0.0036	2.3140	2.2763	2.3522	2.2977	2.3305	2.1751	2.4528
16	10-15-24	2.3096	0.3635	0.3645	0.0035	2.3145	2.2775	2.3522	2.2985	2.3308	2.1756	2.4534
17	10-22-24	2.3096	0.3635	0.3645	0.0035	2.3145	2.2774	2.3522	2.2985	2.3308	2.1756	2.4534
18	11-05-24	2.2980	0.3613	0.3642	0.0036	2.3133	2.2757	2.3515	2.2970	2.3298	2.1745	2.4521
19	11-13-24	2.3099	0.3636	0.3642	0.0036	2.3133	2.2757	2.3515	2.2970	2.3298	2.1745	2.4521
20	12-03-24	2.2868	0.3592	0.3637	0.0034	2.3104	2.2741	2.3473	2.2947	2.3263	2.1718	2.4490

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC_{50} values.

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

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

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.10}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Kim 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 # 420

Dilution Preparation:

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

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

Stock solution INSS #: 2331

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	VL	Em	Em
Control, MHSW	pH (S.U.)	7.06	7.41	7.36
	Dissolved oxygen (mg/L)	7.6	7.8	7.9
	Conductivity (µmhos/cm)	292		
	Alkalinity (mg/L $CaCO_3$)	59		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	24.9	25.3	25.2
1750 mg/L	pH (S.U.)	7.70	7.42	7.47
	Dissolved oxygen (mg/L)	8.0	7.7	7.9
	Conductivity (µmhos/cm)	3330		
	Temperature (°C)	25.1	25.0	25.2
2000 mg/L	pH (S.U.)	7.76	7.43	7.48
	Dissolved oxygen (mg/L)	8.0	7.7	7.9
	Conductivity (µmhos/cm)	3780		
	Temperature (°C)	25.0	25.0	25.0
2250 mg/L	pH (S.U.)	7.76	7.45	7.49
	Dissolved oxygen (mg/L)	8.0	7.7	8.0
	Conductivity (µmhos/cm)	4210		
	Temperature (°C)	25.0	25.2	25.0
2500 mg/L	pH (S.U.)	7.75	7.46	7.49
	Dissolved oxygen (mg/L)	8.0	7.7	8.0
	Conductivity (µmhos/cm)	4090		
	Temperature (°C)	25.0	25.2	24.9
2750 mg/L	pH (S.U.)	7.75	7.50	7.50
	Dissolved oxygen (mg/L)	8.1	7.8	8.0
	Conductivity (µmhos/cm)	5100		
	Temperature (°C)	25.2	25.0	25.3

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 numbe
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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	13066465

ETS

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 # 420

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	12-03-24	* 0530	JL	0745	JL	2B2	RED	11-27-24A
24	12-04-24			0740	JL			
48 Termination	12-05-24			0740	JL			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	11-26-24 D
Age:	< 24-hours old
Date and time organisms were born between:	12-02-24 1500 TO 12-03-24 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.62
	Temperature (°C): 25.0

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

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	4 1d	4 1d	4 1d	5	3 2d	2 3d	4 1d	2 3d
48 Termination	3 2d	1 4d	2 3d	3 2d	1 3d	1 3d	1 3d	1 4d	0 3d	0 2d	0 4d	0 2d
Mean Survival	45%				20%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2212.7
Upper 95% confidence limit (mg NaCl/L)	2359.1
48-hour LC_{50} (mg NaCl/L)	2286.8

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	12/3/2024	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	12/5/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

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

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.4500	0.4500	0.7301	0.4636	0.8861	27.587	4	10.00	10.00	11	20
*2500	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	4	10.00	10.00	16	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

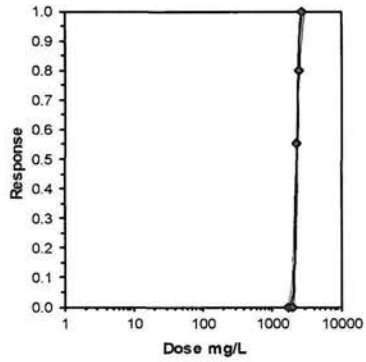
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.57538	0.868	-1.3039	7.42774
Equality of variance cannot be confirmed				

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

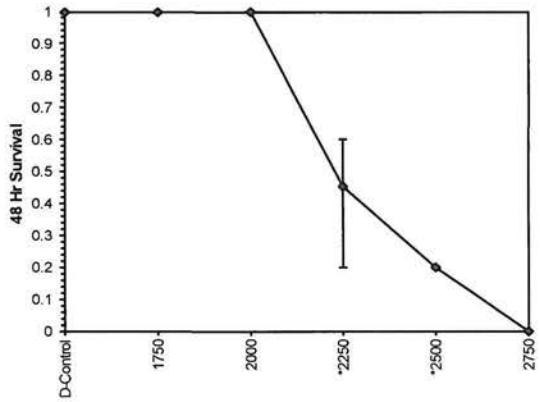
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	30.009	5.43229	19.3617	40.6563	0	3.59118	7.81472	0.30913	3.35923	0.03332	6
Intercept	-95.807	18.2649	-131.61	-60.008							
TSCR											

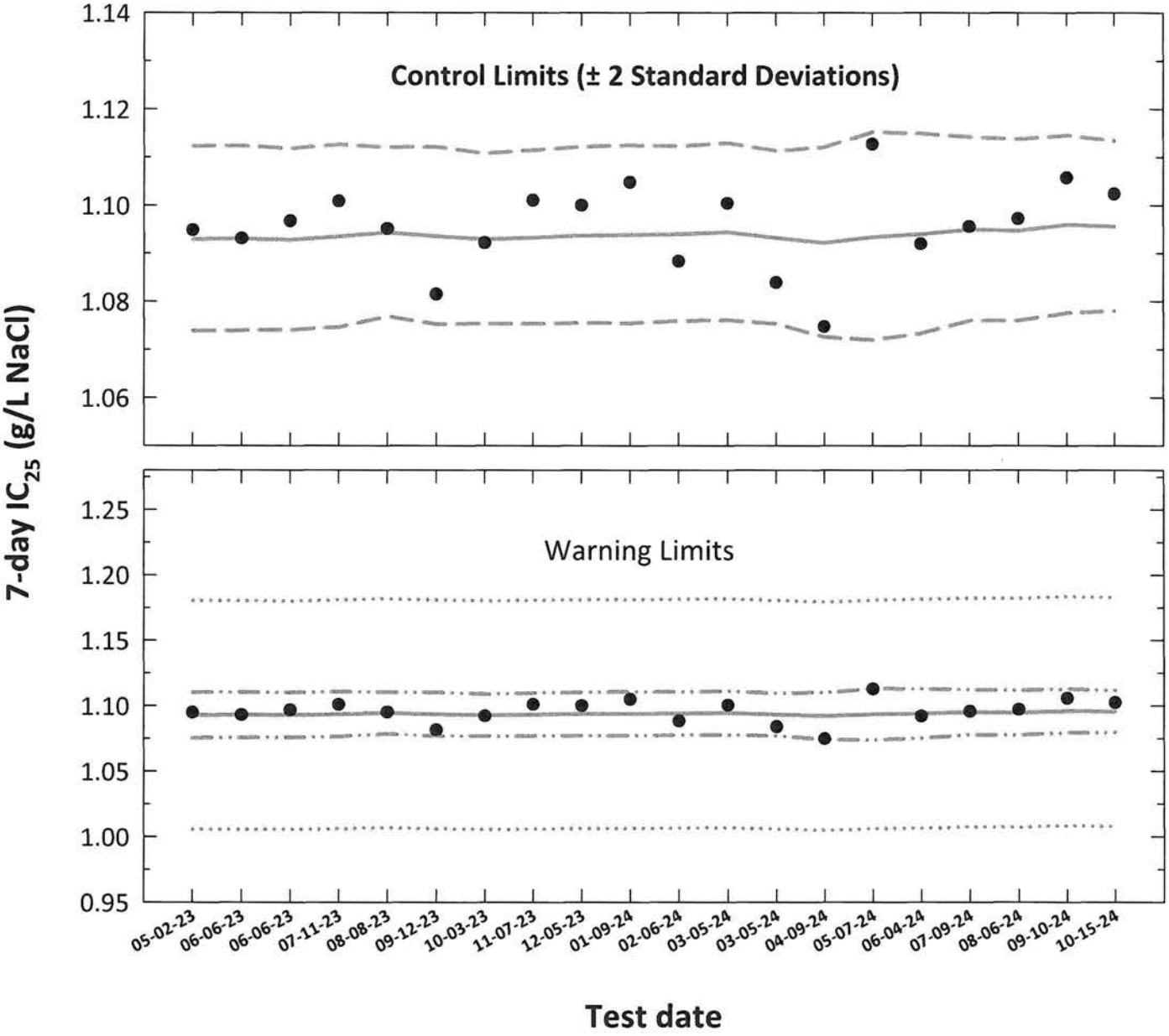
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1912.99	1716.7	2021.08
EC05	3.355	2015.69	1857.45	2105.33
EC10	3.718	2072.67	1935.8	2153.18
EC15	3.964	2112.02	1989.57	2187.1
EC20	4.158	2143.82	2032.58	2215.31
EC25	4.326	2171.49	2069.44	2240.63
EC40	4.747	2242.81	2160.55	2310.84
EC50	5.000	2286.83	2212.65	2359.06
EC60	5.253	2331.72	2261.69	2412.87
EC75	5.674	2408.3	2336.4	2514.95
EC80	5.842	2439.39	2364.18	2559.41
EC85	6.036	2476.12	2395.65	2613.67
EC90	6.282	2523.13	2434.3	2685.27
EC95	6.645	2594.46	2490.51	2797.52
EC99	7.326	2733.73	2595.25	3025.82



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

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	05-02-23	1.0948	0.0393	0.0386	0.0038	1.0929	1.0739	1.1123	1.0755	1.1104	1.0055	1.1804
2	06-06-23	1.0931	0.0387	0.0386	0.0038	1.0930	1.0740	1.1124	1.0756	1.1105	1.0056	1.1805
3	06-06-23	1.0967	0.0401	0.0385	0.0037	1.0928	1.0741	1.1118	1.0757	1.1099	1.0054	1.1802
4	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
5	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
6	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
7	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	1.0055	1.1804
8	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753	1.1115	1.0769	1.1097	1.0058	1.1807
9	12-05-23	1.1000	0.0414	0.0389	0.0036	1.0937	1.0756	1.1122	1.0772	1.1103	1.0062	1.1812
10	01-09-24	1.1048	0.0433	0.0389	0.0037	1.0938	1.0755	1.1125	1.0771	1.1106	1.0063	1.1813
11	02-06-24	1.0884	0.0368	0.0390	0.0036	1.0940	1.0760	1.1123	1.0775	1.1105	1.0065	1.1815
12	03-05-24	1.1004	0.0416	0.0392	0.0036	1.0944	1.0761	1.1129	1.0777	1.1110	1.0068	1.1819
13	03-05-24	1.0839	0.0350	0.0387	0.0036	1.0932	1.0754	1.1113	1.0769	1.1095	1.0058	1.1807
14	04-09-24	1.0748	0.0313	0.0383	0.0039	1.0922	1.0726	1.1121	1.0743	1.1101	1.0048	1.1796
15	05-07-24	1.1127	0.0464	0.0388	0.0043	1.0934	1.0720	1.1152	1.0738	1.1130	1.0059	1.1809
16	06-04-24	1.0920	0.0382	0.0390	0.0041	1.0940	1.0734	1.1150	1.0752	1.1128	1.0065	1.1815
17	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
18	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
19	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
20	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

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

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

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

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

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

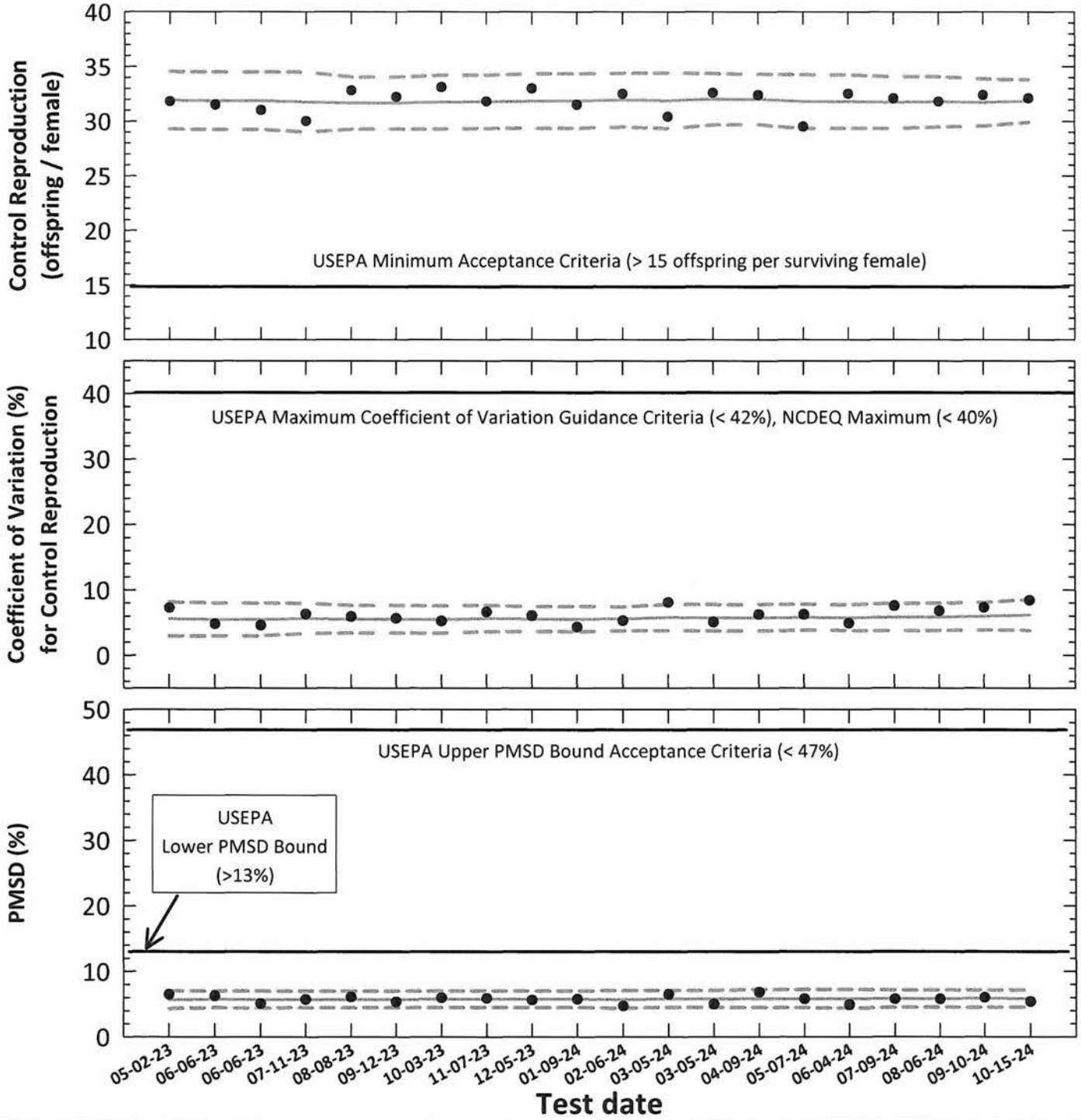
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control Survival (%)	Control Reproduction 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	05-02-23	100	31.8	7.2	2.054	6.5	31.9	29.3	34.5	5.5	2.9	8.2	5.7	4.3	7.0
2	06-06-23	100	31.5	4.8	1.965	6.2	31.9	29.2	34.5	5.4	2.9	8.0	5.7	4.5	7.0
3	06-06-23	100	31.0	4.6	1.573	5.1	31.9	29.2	34.5	5.5	2.9	8.0	5.7	4.4	7.0
4	07-11-23	100	30.0	6.3	1.710	5.7	31.8	29.0	34.5	5.6	3.3	7.9	5.7	4.4	7.0
5	08-08-23	100	32.8	5.9	1.990	6.1	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
6	09-12-23	100	32.2	5.6	1.717	5.3	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
7	10-03-23	100	33.1	5.2	1.970	6.0	31.8	29.3	34.2	5.5	3.4	7.5	5.7	4.5	7.0
8	11-07-23	100	31.8	6.6	1.867	5.9	31.8	29.3	34.2	5.6	3.6	7.6	5.8	4.5	7.0
9	12-05-23	100	33.0	6.1	1.849	5.6	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
10	01-09-24	100	31.5	4.3	1.810	5.7	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
11	02-06-24	100	32.5	5.3	1.526	4.7	31.9	29.5	34.4	5.6	3.7	7.4	5.7	4.4	7.0
12	03-05-24	100	30.4	8.1	1.973	6.5	31.9	29.3	34.4	5.8	3.8	7.8	5.8	4.5	7.1
13	03-05-24	100	32.6	5.1	1.629	5.0	32.0	29.7	34.4	5.7	3.7	7.8	5.8	4.5	7.1
14	04-09-24	100	32.4	6.2	2.199	6.8	32.0	29.7	34.3	5.7	3.7	7.8	5.8	4.4	7.2
15	05-07-24	100	29.5	6.2	1.707	5.8	31.8	29.3	34.3	5.8	3.8	7.8	5.8	4.4	7.2
16	06-04-24	100	32.5	4.9	1.587	4.9	31.8	29.3	34.2	5.7	3.8	7.7	5.8	4.3	7.2
17	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
18	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
19	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
20	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

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

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

CV = Coefficient of variation for control reproduction.
USEPA maximum CV guidance criteria (90th percentile) < 42%. NCDEQ maximum CV acceptance criteria < 40%.

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 13%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 47%.

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 303

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 7320				
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:	10-15-24 0500 TO 0710									
Culture board:	10-08-24 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	3	5	10	11	12	17	18	24	28	29
Transfer vessel information:	pH (S.U.): 7.81 Temperature (°C): 24.9									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

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

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	10-15-24	0730	09-27-24	09-27-24	10-13-24A	[illegible]
1	10-16-24	0635	↓	↓	↓	[illegible]
2	10-17-24	0632				[illegible]
3	10-18-24	0630				[illegible]
4	10-19-24	0636				[illegible]
5	10-20-24	0630				[illegible]
6	10-21-24	0630	↓	↓	↓	[illegible]
7	10-22-24	0631				[illegible]

*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 G-2021	YSI Model 52CE	08A100271
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)	1095.5
% CV:	8.4%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1102.4

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 303**

CONTROL ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	5	4	5	5	4	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	10	13	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	14	14	16	18	19	14	15	14	17	19
Total young produced		32	30	30	36	37	30	31	29	33	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	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	4	6	4	5	4	5	5	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	13	13	10	10	11	12	11	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	18	15	14	16	19	16	15	15	17
Total young produced		31	37	32	32	30	34	32	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:	32.4
% Reduction from Control:	-0.9%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 303**

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.0
% Reduction from Control:	0.3%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.2
% Reduction from Control:	2.8%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 303**

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.5
% Reduction from Control:	45.5%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2.5
% Reduction from Control:	92.2%

Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	4	5	5	4	4	4	4	4	44
5	13	11	10	13	13	12	12	11	12	10	117
6	0	0	0	0	0	0	0	0	0	0	0
7	14	14	16	18	19	14	15	14	17	19	160
Total	**32**	**30**	**30**	**36**	**37**	**30**	**31**	**29**	**33**	**33**	**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	4	5	4	4	4	4	5	3	5	5	43
5	11	10	11	13	10	10	10	13	12	10	110
6	0	0	0	0	0	0	0	0	0	0	0
7	15	16	15	14	17	19	18	15	15	15	159
Total	**30**	**31**	**30**	**31**	**31**	**33**	**33**	**31**	**32**	**30**	**312**

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	5	4	5	5	5	5	5	48
5	12	13	13	13	10	10	11	12	11	11	116
6	0	0	0	0	0	0	0	0	0	0	0
7	15	18	15	14	16	19	16	15	15	17	160
Total	**31**	**37**	**32**	**32**	**30**	**34**	**32**	**32**	**31**	**33**	**324**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	3	2	3	3	3	4	4	4	33
5	7	9	6	11	9	5	8	9	11	7	82
6	0	0	0	0	0	0	0	0	0	0	0
7	5	6	7	5	6	10	7	6	3	5	60
Total	**15**	**19**	**16**	**18**	**18**	**18**	**18**	**19**	**18**	**16**	**175**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	5	5	5	4	5	4	4	6	46
5	11	12	12	13	11	12	12	10	12	10	115
6	0	0	0	0	0	0	0	0	0	0	0
7	17	16	17	13	14	18	15	18	17	14	159
Total	**33**	**31**	**34**	**31**	**30**	**34**	**32**	**32**	**33**	**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	2	2	3	1	1	1	1	2	16
5	0	1	0	0	0	2	0	0	2	0	5
6	0	0	0	0	0	0	0	1	0	0	1
7	0	0	0	2	0	0	1	0	0	0	3
Total	**2**	**2**	**2**	**4**	**3**	**3**	**2**	**2**	**3**	**2**	**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: 303

Test dates: October 15-22, 2024

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	32	30	30	36	37	30	31	29	33	33	**100**	**32.1**	**8.4**	**Not applicable**
600	31	37	32	32	30	34	32	32	31	33	**100**	**32.4**	**6.0**	**-0.9**
800	33	31	34	31	30	34	32	32	33	30	**100**	**32.0**	**4.7**	**0.3**
1000	30	31	30	31	31	33	33	31	32	30	**100**	**31.2**	**3.6**	**2.8**
1200	15	19	16	18	18	18	18	19	18	16	**100**	**17.5**	**7.7**	**45.5**
1400	2	2	2	4	3	3	2	2	3	2	**100**	**2.5**	**28.3**	**92.2**

Dunnett's MSD value: 1.715

PMSD: 5.3

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Summer

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	10/15/2024	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	10/22/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	32.000	30.000	30.000	36.000	37.000	30.000	31.000	29.000	33.000	33.000
600	31.000	37.000	32.000	32.000	30.000	34.000	32.000	32.000	31.000	33.000
800	33.000	31.000	34.000	31.000	30.000	34.000	32.000	32.000	33.000	30.000
1000	30.000	31.000	30.000	31.000	31.000	33.000	33.000	31.000	32.000	30.000
1200	15.000	19.000	16.000	18.000	18.000	18.000	18.000	19.000	18.000	16.000
1400	2.000	2.000	2.000	4.000	3.000	3.000	2.000	2.000	3.000	2.000

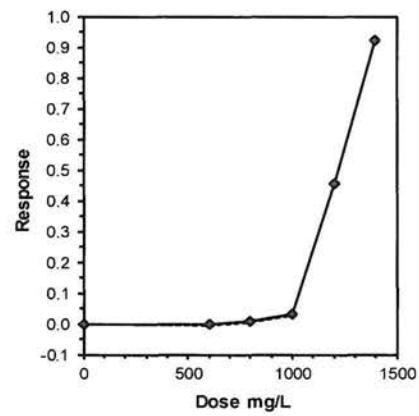
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	32.100	1.0000	32.100	29.000	37.000	8.366	10			32.250	1.0000
600	32.400	1.0093	32.400	30.000	37.000	6.034	10	113.00	75.00	32.250	1.0000
800	32.000	0.9969	32.000	30.000	34.000	4.658	10	110.00	75.00	32.000	0.9922
1000	31.200	0.9720	31.200	30.000	33.000	3.639	10	100.00	75.00	31.200	0.9674
*1200	17.500	0.5452	17.500	15.000	19.000	7.737	10	55.00	75.00	17.500	0.5426
*1400	2.500	0.0779	2.500	2.000	4.000	28.284	10	55.00	75.00	2.500	0.0775

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.74011	1.035	0.83427	1.42028
Bartlett's Test indicates unequal variances (p = 5.33E-03)	16.5962	15.0863		

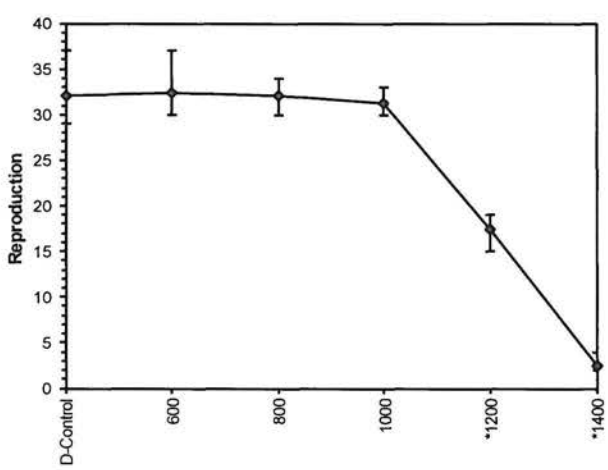
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1000	1200	1095.45	
Treatments vs D-Control				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1008.21	53.5164	839.154	1021.87	-4.2253
IC10	1031.75	7.52069	1015.8	1044.61	-0.4062
IC15	1055.29	6.98933	1040.25	1067.15	-0.3799
IC20	1078.83	6.53874	1064.7	1089.69	-0.3351
IC25	1102.37	6.1866	1089.02	1112.89	-0.2688
IC40	1172.99	5.8752	1160.27	1184.15	0.0055
IC50	1218.33	5.05954	1207.07	1227.23	-0.1096



Dose-Response Plot



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:	10/15/2024	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	10/22/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

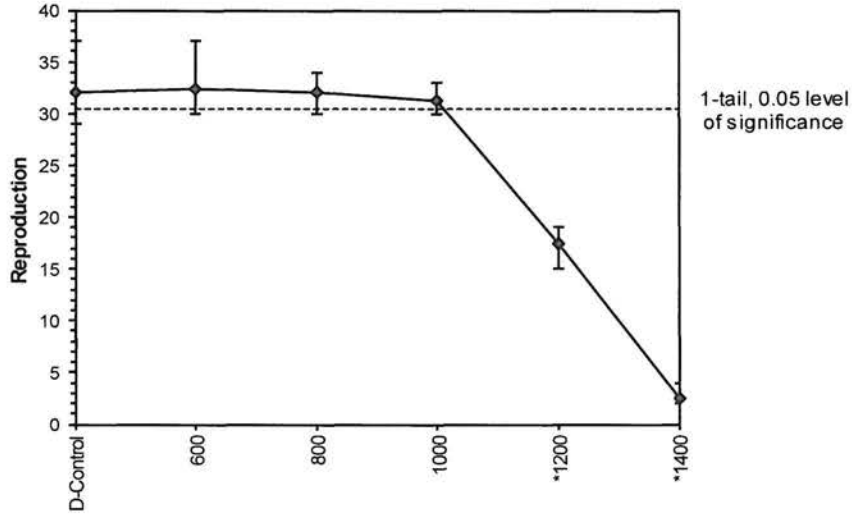
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	32.000	30.000	30.000	36.000	37.000	30.000	31.000	29.000	33.000	33.000
600	31.000	37.000	32.000	32.000	30.000	34.000	32.000	32.000	31.000	33.000
800	33.000	31.000	34.000	31.000	30.000	34.000	32.000	32.000	33.000	30.000
1000	30.000	31.000	30.000	31.000	31.000	33.000	33.000	31.000	32.000	30.000
1200	15.000	19.000	16.000	18.000	18.000	18.000	18.000	19.000	18.000	16.000
1400	2.000	2.000	2.000	4.000	3.000	3.000	2.000	2.000	3.000	2.000

			Transform: Untransformed						1-Tailed	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	32.100	1.0000	32.100	29.000	37.000	8.366	10			
600	32.400	1.0093	32.400	30.000	37.000	6.034	10	-0.400	2.287	1.715
800	32.000	0.9969	32.000	30.000	34.000	4.658	10	0.133	2.287	1.715
1000	31.200	0.9720	31.200	30.000	33.000	3.639	10	1.200	2.287	1.715
*1200	17.500	0.5452	17.500	15.000	19.000	7.737	10	19.465	2.287	1.715
*1400	2.500	0.0779	2.500	2.000	4.000	28.284	10	39.463	2.287	1.715

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.74011	1.035	0.83427	1.42028
Bartlett's Test indicates unequal variances (p = 5.33E-03)	16.5962	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		1.71514	0.05343	1508.46	2.81296	9.8E-45	5, 54
Treatments vs D-Control										

Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 303**

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.

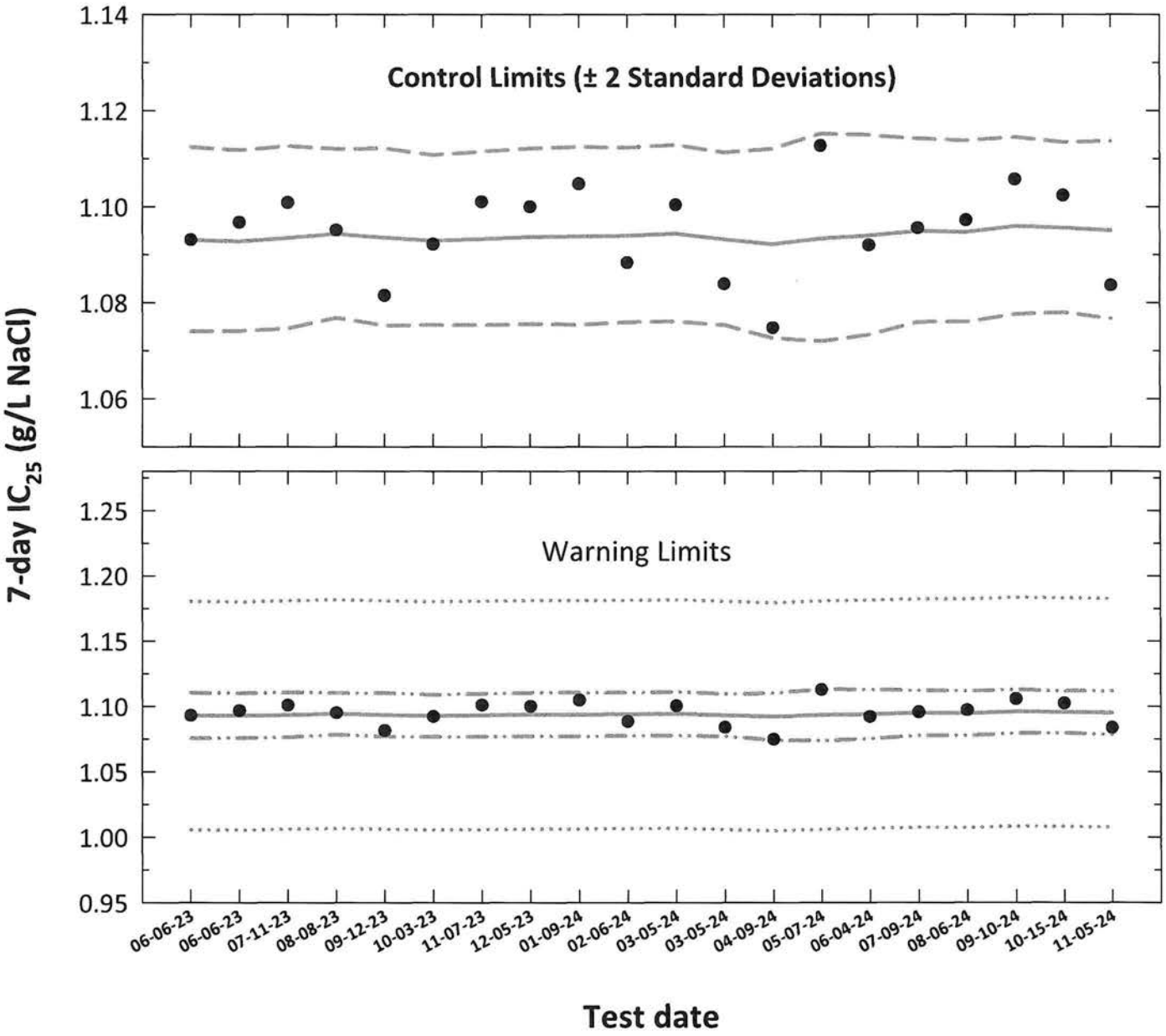
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	M	M	M	EM	EM	M
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.75	7.61	7.54	7.36	7.20	7.74
	Dissolved oxygen (mg/L)	7.9	7.9	7.7	7.9	7.8	7.8
	Conductivity (µmhos/cm)	310		309		298	
	Alkalinity (mg $CaCO_3$/L)	61					
	Hardness (mg $CaCO_3$/L)	88				M 10-19	24 M
	Temperature (°C)	24.9	25.3	24.8	25.3	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.88	7.60	7.66	7.44	7.38	7.73
	Dissolved oxygen (mg/L)	7.8	7.9	7.7	7.9	7.8	7.8
	Conductivity (µmhos/cm)	1410		1450		1470	
	Temperature (°C)	25.0	25.0	24.9	25.0	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.88	7.61	7.66	7.46	7.40	7.72
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	8.0	7.7	7.8
	Conductivity (µmhos/cm)	1790		1820		1850	
	Temperature (°C)	25.0	25.0	24.9	25.2	25.0	25.0
1000 mg NaCl/L	pH (S.U.)	7.88	7.60	7.66	7.49	7.44	7.72
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	8.0	7.7	7.8
	Conductivity (µmhos/cm)	2170		2220		2240	
	Temperature (°C)	25.1	25.2	24.9	25.2	25.0	24.9
1200 mg NaCl/L	pH (S.U.)	7.88	7.60	7.66	7.48	7.46	7.73
	Dissolved oxygen (mg/L)	7.9	7.8	7.8	7.9	7.8	7.8
	Conductivity (µmhos/cm)	2520		2570		2580	
	Temperature (°C)	25.0	25.2	24.8	25.2	25.0	24.9
1400 mg NaCl/L	pH (S.U.)	7.89	7.60	7.66	7.48	7.46	7.71
	Dissolved oxygen (mg/L)	7.9	7.8	7.8	7.9	7.8	7.8
	Conductivity (µmhos/cm)	2900		2940		2970	
	Temperature (°C)	25.0	25.1	24.8	25.1	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 303**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	K	K	K	K	K	K	K	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.80	7.66	7.70	7.64	7.68	7.66	7.73	7.42
	Dissolved oxygen (mg/L)	8.0	8.0	8.0	7.8	8.0	7.9	7.8	7.7
	Conductivity (µmhos/cm)	298		307		305		310	
	Alkalinity (mg $CaCO_3$/L)								
	Hardness (mg $CaCO_3$/L)							H 10-19-24 K	
	Temperature (°C)	24.9	25.2	24.8	25.1	24.8	25.3	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.81	7.68	7.75	7.67	7.75	7.71	7.77	7.42
	Dissolved oxygen (mg/L)	8.0	8.0	8.0	7.8	8.0	7.8	7.8	7.7
	Conductivity (µmhos/cm)	1360		1380		1400		1420	
	Temperature (°C)	24.9	25.0	24.7	24.9	24.9	25.0	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.78	7.68	7.74	7.67	7.74	7.72	7.78	7.51
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.8	8.0	7.9	7.7	7.8
	Conductivity (µmhos/cm)	1710		1760		1760		1810	
	Temperature (°C)	25.0	24.9	24.7	24.9	24.9	25.0	24.9	25.2
1000 mg NaCl/L	pH (S.U.)	7.78	7.68	7.75	7.67	7.74	7.72	7.78	7.52
	Dissolved oxygen (mg/L)	8.0	7.9	8.0	7.8	8.0	7.8	7.8	7.8
	Conductivity (µmhos/cm)	2080		2100		2100		2130	
	Temperature (°C)	25.0	24.9	24.7	24.9	24.9	25.0	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	7.78	7.68	7.75	7.66	7.75	7.70	7.77	7.52
	Dissolved oxygen (mg/L)	8.0	7.9	7.9	7.7	8.0	7.7	7.8	7.8
	Conductivity (µmhos/cm)	2410		2430		2470		2500	
	Temperature (°C)	25.0	25.2	24.9	25.1	25.0	25.2	25.0	25.2
1400 mg NaCl/L	pH (S.U.)	7.77	7.69	7.75	7.66	7.75	7.70	7.77	7.55
	Dissolved oxygen (mg/L)	8.0	7.9	7.9	7.7	8.0	7.7	7.9	7.8
	Conductivity (µmhos/cm)	2760		2780		2830		2850	
	Temperature (°C)	25.0	24.9	24.7	24.9	25.0	25.1	25.0	25.2
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	06-06-23	1.0931	0.0387	0.0386	0.0038	1.0930	1.0740	1.1124	1.0756	1.1105	1.0056	1.1805
2	06-06-23	1.0967	0.0401	0.0385	0.0037	1.0928	1.0741	1.1118	1.0757	1.1099	1.0054	1.1802
3	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
4	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
5	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
6	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	1.0055	1.1804
7	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753	1.1115	1.0769	1.1097	1.0058	1.1807
8	12-05-23	1.1000	0.0414	0.0389	0.0036	1.0937	1.0756	1.1122	1.0772	1.1103	1.0062	1.1812
9	01-09-24	1.1048	0.0433	0.0389	0.0037	1.0938	1.0755	1.1125	1.0771	1.1106	1.0063	1.1813
10	02-06-24	1.0884	0.0368	0.0390	0.0036	1.0940	1.0760	1.1123	1.0775	1.1105	1.0065	1.1815
11	03-05-24	1.1004	0.0416	0.0392	0.0036	1.0944	1.0761	1.1129	1.0777	1.1110	1.0068	1.1819
12	03-05-24	1.0839	0.0350	0.0387	0.0036	1.0932	1.0754	1.1113	1.0769	1.1095	1.0058	1.1807
13	04-09-24	1.0748	0.0313	0.0383	0.0039	1.0922	1.0726	1.1121	1.0743	1.1101	1.0048	1.1796
14	05-07-24	1.1127	0.0464	0.0388	0.0043	1.0934	1.0720	1.1152	1.0738	1.1130	1.0059	1.1809
15	06-04-24	1.0920	0.0382	0.0390	0.0041	1.0940	1.0734	1.1150	1.0752	1.1128	1.0065	1.1815
16	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
17	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
18	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
19	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
20	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

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

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

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

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

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

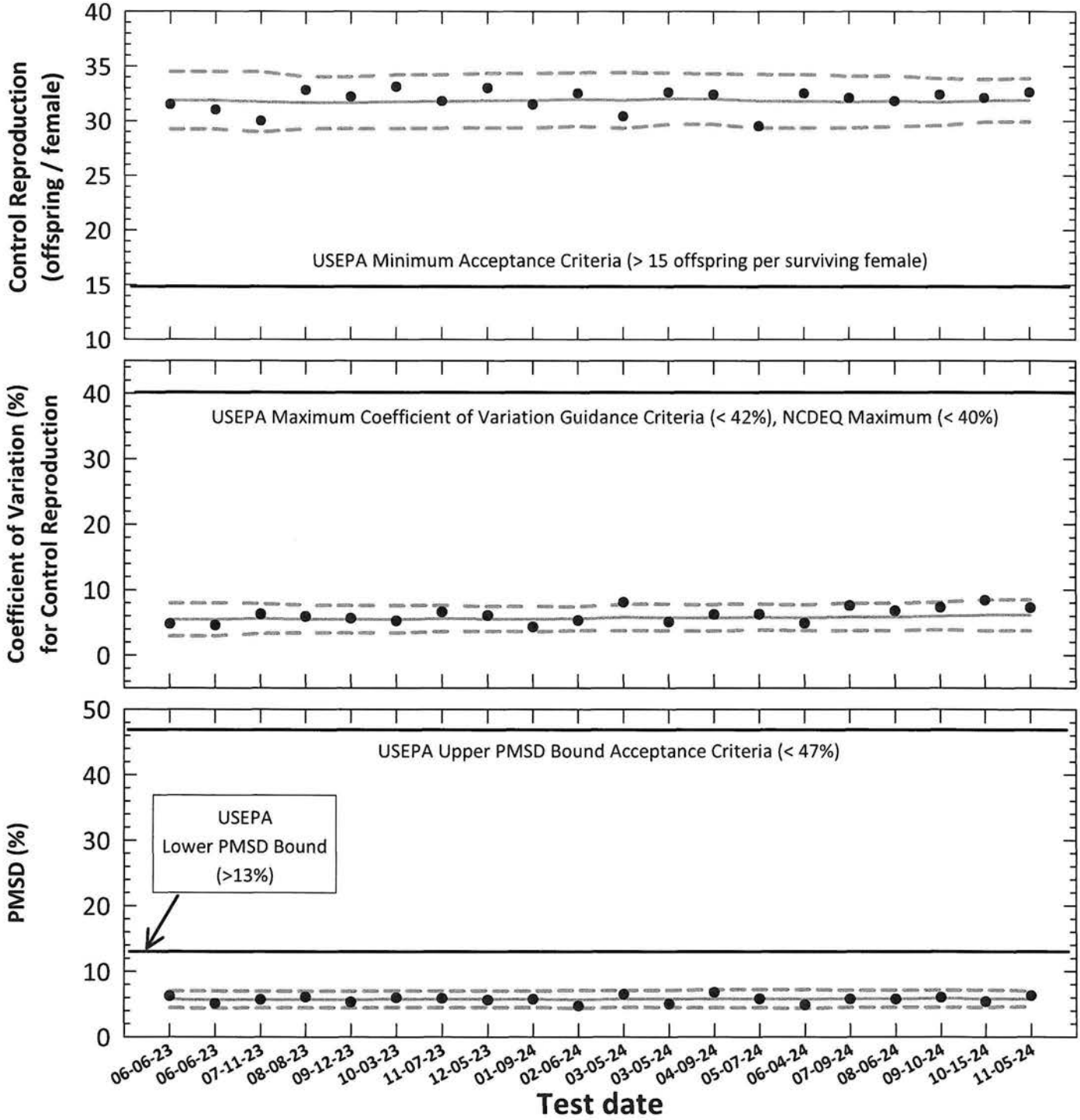
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control Survival (%)	Control Reproduction 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	06-06-23	100	31.5	4.8	1.965	6.2	31.9	29.2	34.5	5.4	2.9	8.0	5.7	4.5	7.0
2	06-06-23	100	31.0	4.6	1.573	5.1	31.9	29.2	34.5	5.5	2.9	8.0	5.7	4.4	7.0
3	07-11-23	100	30.0	6.3	1.710	5.7	31.8	29.0	34.5	5.6	3.3	7.9	5.7	4.4	7.0
4	08-08-23	100	32.8	5.9	1.990	6.1	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
5	09-12-23	100	32.2	5.6	1.717	5.3	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
6	10-03-23	100	33.1	5.2	1.970	6.0	31.8	29.3	34.2	5.5	3.4	7.5	5.7	4.5	7.0
7	11-07-23	100	31.8	6.6	1.867	5.9	31.8	29.3	34.2	5.6	3.6	7.6	5.8	4.5	7.0
8	12-05-23	100	33.0	6.1	1.849	5.6	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
9	01-09-24	100	31.5	4.3	1.810	5.7	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
10	02-06-24	100	32.5	5.3	1.526	4.7	31.9	29.5	34.4	5.6	3.7	7.4	5.7	4.4	7.0
11	03-05-24	100	30.4	8.1	1.973	6.5	31.9	29.3	34.4	5.8	3.8	7.8	5.8	4.5	7.1
12	03-05-24	100	32.6	5.1	1.629	5.0	32.0	29.7	34.4	5.7	3.7	7.8	5.8	4.5	7.1
13	04-09-24	100	32.4	6.2	2.199	6.8	32.0	29.7	34.3	5.7	3.7	7.8	5.8	4.4	7.2
14	05-07-24	100	29.5	6.2	1.707	5.8	31.8	29.3	34.3	5.8	3.8	7.8	5.8	4.4	7.2
15	06-04-24	100	32.5	4.9	1.587	4.9	31.8	29.3	34.2	5.7	3.8	7.7	5.8	4.3	7.2
16	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
17	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
18	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
19	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
20	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

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

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

CV = Coefficient of variation for control reproduction.
USEPA maximum CV guidance criteria (90th percentile) < 42%. NCDEQ maximum CV acceptance criteria < 40%.

MSD = Minimum significant difference.

PMSD = Percent minimum significant difference.
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test.
Lower PMSD bound determined by USEPA (10th percentile) > 13%.
The lower PMSD bound represents a practical limit to the sensitivity of the test method and is not a minimum acceptance criteria.
Upper PMSD bound acceptance criteria determined by USEPA (90th percentile) < 47%.

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

CdNaClCR #: 304

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2331				
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:	11-05-24 0415 TO 0810									
Culture board:	10-29-24 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	2	3	8	11	12	15	17	22	23
Transfer vessel information:	pH (S.U.): 7.74 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	BLUE
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	11-05-24	0822	10-31-24	10-31-24	10-30-24 A	JH
1	11-06-24	0623	↓	↓	↓	JH
2	11-07-24	0622	↓	↓	10-30-24 B	JH
3	11-08-24	0624	↓	↓	↓	JH
4	11-09-24	0800	↓	↓	11-04-24 B	JH
5	11-10-24	0622	↓	↓	↓	JH
6	11-11-24	0623	↓	↓	↓	JH
7	11-12-24	0635				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 G-2021	YSI Model 52CE	08A100271
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:	32.6	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.4
% CV:	7.3%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1083.7

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 304**

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	5	3	3	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	12	12	10	13	11	13	13	12	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	15	18	15	19	17	19	14	15	19
Total young produced		31	31	34	30	35	31	36	31	31	36
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:	32.6

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	4	3	4	4	4	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	13	13	11	11	12	11	13	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	20	19	18	16	17	19	19	14	16
Total young produced		32	34	36	34	31	32	35	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:	33.3
% Reduction from Control:	-2.1%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 304**

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	3	5	4	4	4	5	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	9	10	10	12	12	12	12	10	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	19	17	16	18	19	18	19	19	15	15
Total young produced		35	SB 14 29	31	32	35	34	36	36	29	30
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.7
% Reduction from Control:	−0.3%

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	6	4	4	4	4	5	3	3	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	9	10	12	10	10	11	12	10	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	17	14	16	13	16	15	15	15	14
Total young produced		33	30	28	32	27	31	29	30	29	30
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	29.9
% Reduction from Control:	8.3%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 304**

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	5	3	3	3	4	3	5	5	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	5	10	5	8	7	11	6	8	9
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	4	10	6	5	8	5	5	6	5	5
Total young produced		17	20	19	13	19	16	19	17	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:	17.5
% Reduction from Control:	46.3%

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	1.9
% Reduction from Control:	94.2%

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	5	3	3	4	4	4	4	40
5	10	12	12	10	13	11	13	13	12	13	119
6	0	0	0	0	0	0	0	0	0	0	0
7	16	15	18	15	19	17	19	14	15	19	167
Total	**31**	**31**	**34**	**30**	**35**	**31**	**36**	**31**	**31**	**36**	**326**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	6	4	4	4	4	5	3	3	4	4	41
5	10	9	10	12	10	10	11	12	10	12	106
6	0	0	0	0	0	0	0	0	0	0	0
7	17	17	14	16	13	16	15	15	15	14	152
Total	**33**	**30**	**28**	**32**	**27**	**31**	**29**	**30**	**29**	**30**	**299**

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	4	3	4	4	4	5	5	4	42
5	12	10	13	13	11	11	12	11	13	12	118
6	0	0	0	0	0	0	0	0	0	0	0
7	15	20	19	18	16	17	19	19	14	16	173
Total	**32**	**34**	**36**	**34**	**31**	**32**	**35**	**35**	**32**	**32**	**333**

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	5	3	3	3	4	3	5	5	3	38
5	9	5	10	5	8	7	11	6	8	9	78
6	0	0	0	0	0	0	0	0	0	0	0
7	4	10	6	5	8	5	5	6	5	5	59
Total	**17**	**20**	**19**	**13**	**19**	**16**	**19**	**17**	**18**	**17**	**175**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	5	4	4	4	5	5	4	4	42
5	12	9	10	10	12	12	12	12	10	11	110
6	0	0	0	0	0	0	0	0	0	0	0
7	19	17	16	18	19	18	19	19	15	15	175
Total	**35**	**29**	**31**	**32**	**35**	**34**	**36**	**36**	**29**	**30**	**327**

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	0	1	1	3	1	2	2	0	2	14
5	0	2	1	0	0	0	0	0	1	0	4
6	0	0	0	0	0	1	0	0	0	0	1
7	0	0	0	0	0	0	0	0	0	0	0
Total	**2**	**2**	**2**	**1**	**3**	**2**	**2**	**2**	**1**	**2**	**19**

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: 304

Test dates: November 05-12, 2024

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	31	34	30	35	31	36	31	31	36	100	32.6	7.3	Not applicable
600	32	34	36	34	31	32	35	35	32	32	100	33.3	5.1	-2.1
800	35	29	31	32	35	34	36	36	29	30	100	32.7	8.7	-0.3
1000	33	30	28	32	27	31	29	30	29	30	100	29.9	6.0	8.3
1200	17	20	19	13	19	16	19	17	18	17	100	17.5	11.5	46.3
1400	2	2	2	1	3	2	2	2	1	2	100	1.9	29.9	94.2

Dunnett's MSD value: 2.050

PMSD: 6.3

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Surtsoor

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	11/5/2024	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	11/12/2024	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	31.000	34.000	30.000	35.000	31.000	36.000	31.000	31.000	36.000
600	32.000	34.000	36.000	34.000	31.000	32.000	35.000	35.000	32.000	32.000
800	35.000	29.000	31.000	32.000	35.000	34.000	36.000	36.000	29.000	30.000
1000	33.000	30.000	28.000	32.000	27.000	31.000	29.000	30.000	29.000	30.000
1200	17.000	20.000	19.000	13.000	19.000	16.000	19.000	17.000	18.000	17.000
1400	2.000	2.000	2.000	1.000	3.000	2.000	2.000	2.000	1.000	2.000

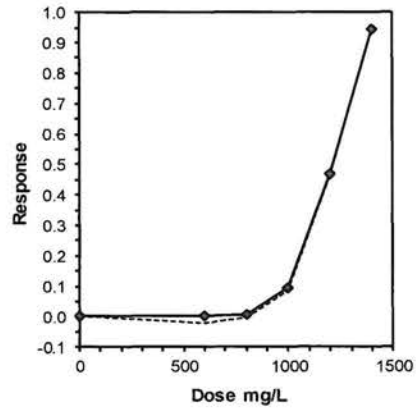
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	32.600	1.0000	32.600	30.000	36.000	7.259	10			32.950	1.0000
600	33.300	1.0215	33.300	31.000	36.000	5.114	10	119.50	75.00	32.950	1.0000
800	32.700	1.0031	32.700	29.000	36.000	8.656	10	104.50	75.00	32.700	0.9924
*1000	29.900	0.9172	29.900	27.000	33.000	5.993	10	72.00	75.00	29.900	0.9074
*1200	17.500	0.5368	17.500	13.000	20.000	11.508	10	55.00	75.00	17.500	0.5311
*1400	1.900	0.0583	1.900	1.000	3.000	29.876	10	55.00	75.00	1.900	0.0577

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.75216	1.035	-0.057	-0.5267
Bartlett's Test indicates unequal variances (p = 3.49E-03)	17.6014	15.0863		

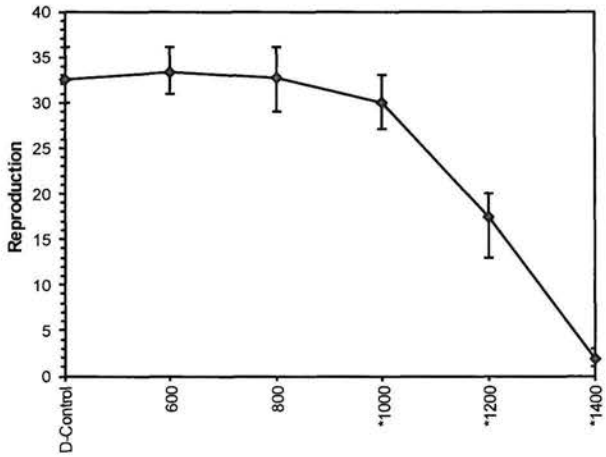
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	800	1000	894.427	
Treatments vs D-Control				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	899.821	50.9674	745.65	973.973	-0.9908
IC10	1003.95	25.606	926.28	1021.61	-1.3659
IC15	1030.52	9.12064	1009.25	1046.95	-0.0690
IC20	1057.1	8.2441	1037.68	1072.1	0.1047
IC25	1083.67	7.78158	1066.27	1097.58	0.1245
IC40	1163.39	8.91311	1143.18	1177.67	-0.1849
IC50	1213.14	7.99165	1194.06	1224.56	-0.6550



Dose-Response Plot



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:	11/5/2024	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	11/12/2024	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:					

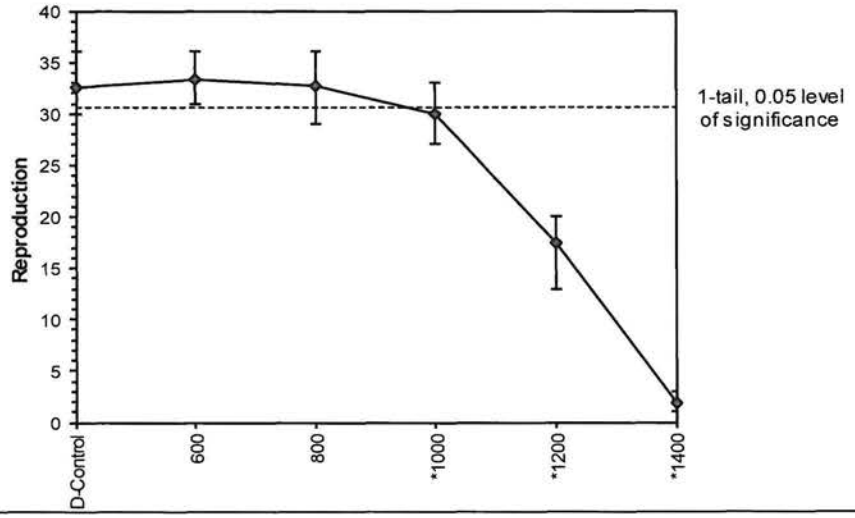
Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	31.000	31.000	34.000	30.000	35.000	31.000	36.000	31.000	31.000	36.000
600	32.000	34.000	36.000	34.000	31.000	32.000	35.000	35.000	32.000	32.000
800	35.000	29.000	31.000	32.000	35.000	34.000	36.000	36.000	29.000	30.000
1000	33.000	30.000	28.000	32.000	27.000	31.000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

			Transform: Untransformed						1-Tailed	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	32.600	1.0000	32.600	30.000	36.000	7.259	10			
600	33.300	1.0215	33.300	31.000	36.000	5.114	10	-0.781	2.287	2.050
800	32.700	1.0031	32.700	29.000	36.000	8.656	10	-0.112	2.287	2.050
*1000	29.900	0.9172	29.900	27.000	33.000	5.993	10	3.012	2.287	2.050
*1200	17.500	0.5368	17.500	13.000	20.000	11.508	10	16.847	2.287	2.050
*1400	1.900	0.0583	1.900	1.000	3.000	29.876	10	34.252	2.287	2.050

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.75216	1.035	-0.057	-0.5267
Bartlett's Test indicates unequal variances (p = 3.49E-03)	17.6014	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	800	1000	894.427		2.04951	0.06287	1598.15	4.01667	2.7E-41	5, 54
Treatments vs D-Control										

Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 304**

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.

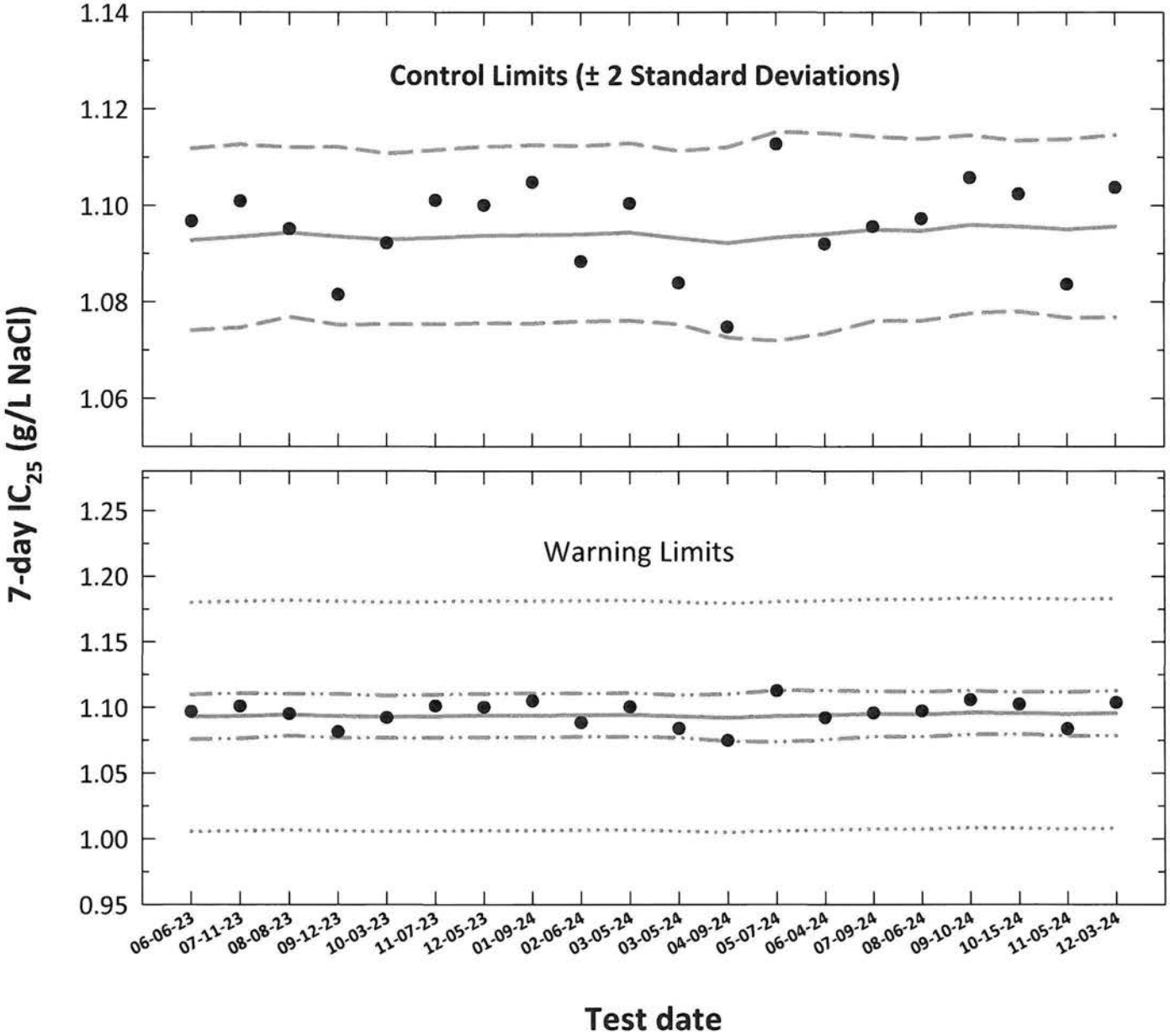
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	N	N	N	EM	EM	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.71	7.75	7.71	7.49	7.51	7.75
	Dissolved oxygen (mg/L)	7.7	7.8	7.7	7.9	7.7	7.7
	Conductivity (µmhos/cm)	298		308		312	
	Alkalinity (mg $CaCO_3$/L)	61				61	
	Hardness (mg $CaCO_3$/L)	92				92	
	Temperature (°C)	24.8	25.2	24.9	25.0	24.8	25.4
600 mg NaCl/L	pH (S.U.)	7.87	7.75	7.81	7.56	7.61	7.80
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.9	7.8	7.6
	Conductivity (µmhos/cm)	1440		1480		1490	
	Temperature (°C)	24.9	25.0	24.9	24.9	24.8	25.2
800 mg NaCl/L	pH (S.U.)	7.90	7.75	7.81	7.57	7.62	7.80
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.9	7.8	7.6
	Conductivity (µmhos/cm)	1780		1910		1880	
	Temperature (°C)	25.0	25.3	25.0	25.2	24.9	25.2
1000 mg NaCl/L	pH (S.U.)	7.90	7.74	7.81	7.58	7.63	7.80
	Dissolved oxygen (mg/L)	7.7	7.6	7.7	7.9	7.8	7.6
	Conductivity (µmhos/cm)	2140		2170		2230	
	Temperature (°C)	25.0	25.1	25.0	25.2	24.9	25.2
1200 mg NaCl/L	pH (S.U.)	7.90	7.74	7.81	7.59	7.63	7.79
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	7.9	7.8	7.7
	Conductivity (µmhos/cm)	2530		2570		2600	
	Temperature (°C)	25.0	25.1	25.0	25.0	25.0	25.2
1400 mg NaCl/L	pH (S.U.)	7.92	7.73	7.81	7.58	7.64	7.79
	Dissolved oxygen (mg/L)	7.8	7.7	7.8	7.9	7.9	7.7
	Conductivity (µmhos/cm)	2970		2930		3000	
	Temperature (°C)	25.0	25.3	25.0	25.2	24.9	25.3
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 304**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	K	K	K	K	K	BM	BM	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.80	7.47	7.58	7.55	7.83	7.40	7.44	7.79
	Dissolved oxygen (mg/L)	7.7	7.9	7.9	7.8	7.9	7.8	7.9	7.8
	Conductivity (µmhos/cm)	296		293		296		316	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.9	25.2	24.9	24.8	24.8	25.1	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.90	7.52	7.48	7.58	7.59	7.54	7.59	7.79
	Dissolved oxygen (mg/L)	7.7	7.9	7.9	7.8	7.7	7.9	7.9	7.8
	Conductivity (µmhos/cm)	1450		1450		1360		1470	
	Temperature (°C)	25.0	24.9	25.0	24.8	24.9	25.0	24.9	25.0
800 mg NaCl/L	pH (S.U.)	7.91	7.51	7.51	7.59	7.60	7.58	7.62	7.79
	Dissolved oxygen (mg/L)	7.7	7.9	7.9	7.8	7.7	7.9	7.9	7.8
	Conductivity (µmhos/cm)	1830		1790		1700		1860	
	Temperature (°C)	25.0	24.9	25.1	25.0	24.9	25.3	24.8	25.3
1000 mg NaCl/L	pH (S.U.)	7.90	7.52	7.51	7.60	7.61	7.58	7.65	7.79
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	7.9	7.7	7.9	7.9	7.7
	Conductivity (µmhos/cm)	2190		2150		2080		2270	
	Temperature (°C)	25.0	25.1	25.0	24.8	25.0	25.1	24.8	25.3
1200 mg NaCl/L	pH (S.U.)	7.92	7.52	7.52	7.60	7.65	7.57	7.66	7.79
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	8.0	7.8	8.0	7.9	7.7
	Conductivity (µmhos/cm)	2540		2500		2420		2660	
	Temperature (°C)	25.0	25.2	25.0	24.7	25.0	25.1	24.8	25.3
1400 mg NaCl/L	pH (S.U.)	7.90	7.52	7.52	7.59	7.65	7.57	7.65	7.77
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	8.0	7.9	8.0	7.9	7.8
	Conductivity (µmhos/cm)	3000		2860		2820		3070	
	Temperature (°C)	25.0	25.0	25.0	24.7	24.9	24.9	25.0	25.0
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- — — — **Control Limits** (mean logarithmic IC_{25} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic IC_{25} ± $S_{A.10}$ 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	06-06-23	1.0967	0.0401	0.0385	0.0037	1.0928	1.0741	1.1118	1.0757	1.1099	1.0054	1.1802
2	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
3	08-08-23	1.0952	0.0395	0.0392	0.0035	1.0943	1.0769	1.1121	1.0784	1.1103	1.0068	1.1819
4	09-12-23	1.0815	0.0340	0.0388	0.0037	1.0935	1.0753	1.1121	1.0768	1.1103	1.0061	1.1810
5	10-03-23	1.0922	0.0383	0.0386	0.0035	1.0929	1.0754	1.1108	1.0769	1.1090	1.0055	1.1804
6	11-07-23	1.1010	0.0418	0.0387	0.0036	1.0933	1.0753	1.1115	1.0769	1.1097	1.0058	1.1807
7	12-05-23	1.1000	0.0414	0.0389	0.0036	1.0937	1.0756	1.1122	1.0772	1.1103	1.0062	1.1812
8	01-09-24	1.1048	0.0433	0.0389	0.0037	1.0938	1.0755	1.1125	1.0771	1.1106	1.0063	1.1813
9	02-06-24	1.0884	0.0368	0.0390	0.0036	1.0940	1.0760	1.1123	1.0775	1.1105	1.0065	1.1815
10	03-05-24	1.1004	0.0416	0.0392	0.0036	1.0944	1.0761	1.1129	1.0777	1.1110	1.0068	1.1819
11	03-05-24	1.0839	0.0350	0.0387	0.0036	1.0932	1.0754	1.1113	1.0769	1.1095	1.0058	1.1807
12	04-09-24	1.0748	0.0313	0.0383	0.0039	1.0922	1.0726	1.1121	1.0743	1.1101	1.0048	1.1796
13	05-07-24	1.1127	0.0464	0.0388	0.0043	1.0934	1.0720	1.1152	1.0738	1.1130	1.0059	1.1809
14	06-04-24	1.0920	0.0382	0.0390	0.0041	1.0940	1.0734	1.1150	1.0752	1.1128	1.0065	1.1815
15	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
16	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
17	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
18	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
19	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
20	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

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

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

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

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

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

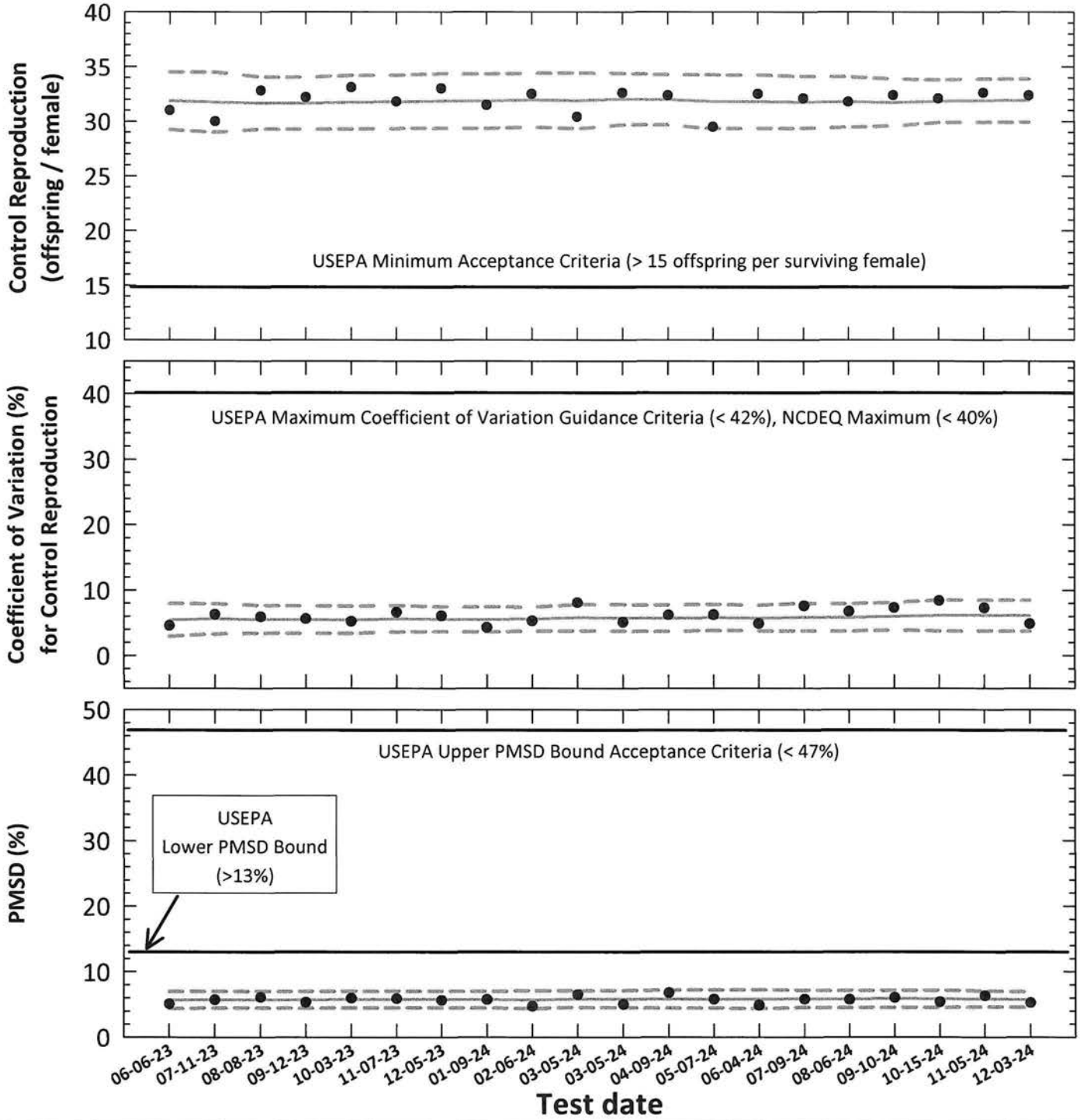
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control Survival (%)	Control Reproduction 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	06-06-23	100	31.0	4.6	1.573	5.1	31.9	29.2	34.5	5.5	2.9	8.0	5.7	4.4	7.0
2	07-11-23	100	30.0	6.3	1.710	5.7	31.8	29.0	34.5	5.6	3.3	7.9	5.7	4.4	7.0
3	08-08-23	100	32.8	5.9	1.990	6.1	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
4	09-12-23	100	32.2	5.6	1.717	5.3	31.7	29.3	34.0	5.5	3.4	7.6	5.7	4.4	7.0
5	10-03-23	100	33.1	5.2	1.970	6.0	31.8	29.3	34.2	5.5	3.4	7.5	5.7	4.5	7.0
6	11-07-23	100	31.8	6.6	1.867	5.9	31.8	29.3	34.2	5.6	3.6	7.6	5.8	4.5	7.0
7	12-05-23	100	33.0	6.1	1.849	5.6	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
8	01-09-24	100	31.5	4.3	1.810	5.7	31.9	29.4	34.4	5.5	3.6	7.4	5.7	4.5	7.0
9	02-06-24	100	32.5	5.3	1.526	4.7	31.9	29.5	34.4	5.6	3.7	7.4	5.7	4.4	7.0
10	03-05-24	100	30.4	8.1	1.973	6.5	31.9	29.3	34.4	5.8	3.8	7.8	5.8	4.5	7.1
11	03-05-24	100	32.6	5.1	1.629	5.0	32.0	29.7	34.4	5.7	3.7	7.8	5.8	4.5	7.1
12	04-09-24	100	32.4	6.2	2.199	6.8	32.0	29.7	34.3	5.7	3.7	7.8	5.8	4.4	7.2
13	05-07-24	100	29.5	6.2	1.707	5.8	31.8	29.3	34.3	5.8	3.8	7.8	5.8	4.4	7.2
14	06-04-24	100	32.5	4.9	1.587	4.9	31.8	29.3	34.2	5.7	3.8	7.7	5.8	4.3	7.2
15	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
16	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
17	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
18	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
19	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
20	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

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 #: 305

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2331				
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:	12-03-24 0530 TO 0755									
Culture board:	11-26-24 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	1	2	3	7	10	11	17	18	23	29
Transfer vessel information:	pH (S.U.): 7.62 Temperature (°C): 25.0									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

Randomizing template color:	RED
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	12-03-24	0806	11-22-24	11-21-24	11-27-24 A	JH
1	12-04-24	0620	↓	↓	↓	JH
2	12-05-24	0622	↓	↓	11-27-24 C	JH
3	12-06-24	0624	↓	↓	↓	JH
4	12-07-24	0610	↓	↓	12-06-24	JH
5	12-08-24	0610	↓	↓	↓	JH
6	12-09-24	0607	↓	↓	↓	JH
7	12-10-24	0610				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 G-2021	YSI Model 52CE	08A100271
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:	32.4	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	4.9%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1103.7

ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 305**

CONTROL ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	6	4	4	5	5	5	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	11	13	10	10	10	10	10	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	18	15	19	17	19	16	16	16	18
Total young produced		31	35	32	33	32	34	31	30	32	34
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:	32.4

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	6	4	4	6	4	5	5	5	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	12	13	10	11	10	10	10	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	14	15	18	19	15	19	19	16	16
Total young produced		33	32	32	32	36	29	34	34	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:	32.6
% Reduction from Control:	-0.6%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 305**

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	4	6	6	4	6	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	11	12	10	12	10	10	10	12	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	17	18	14	13	18	13	17	19	19
Total young produced		35	32	34	30	31	32	29	31	35	35
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.4
% Reduction from Control:	0.0%

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	6	4	4	4	4	4	4	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	12	12	10	10	9	10	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	16	14	15	14	19	16	16	16	16	15
Total young produced		33	32	31	28	33	29	30	31	31	29
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.7
% Reduction from Control:	5.2%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 305**

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	5	4	3	3	3	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	11	8	6	9	5	10	7	6	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	3	7	10	9	10	3	6	8	5
Total young produced		18	19	19	19	21	18	17	17	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:	18.5
% Reduction from Control:	42.9%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	1.9
% Reduction from Control:	94.1%

Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	4	4	5	5	5	4	4	4	45
5	13	11	13	10	10	10	10	10	12	12	111
6	0	0	0	0	0	0	0	0	0	0	0
7	14	18	15	19	17	19	16	16	16	18	168
Total	**31**	**35**	**32**	**33**	**32**	**34**	**31**	**30**	**32**	**34**	**324**

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	6	4	4	4	4	4	4	3	4	42
5	12	12	12	10	10	9	10	11	12	10	108
6	0	0	0	0	0	0	0	0	0	0	0
7	16	14	15	14	19	16	16	16	16	15	157
Total	**33**	**32**	**31**	**28**	**33**	**29**	**30**	**31**	**31**	**29**	**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	5	6	4	4	6	4	5	5	5	4	48
5	13	12	13	10	11	10	10	10	13	10	112
6	0	0	0	0	0	0	0	0	0	0	0
7	15	14	15	18	19	15	19	19	16	16	166
Total	**33**	**32**	**32**	**32**	**36**	**29**	**34**	**34**	**34**	**30**	**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	5	4	3	3	3	4	4	4	4	38
5	9	11	8	6	9	5	10	7	6	10	81
6	0	0	0	0	0	0	0	0	0	0	0
7	5	3	7	10	9	10	3	6	8	5	66
Total	**18**	**19**	**19**	**19**	**21**	**18**	**17**	**17**	**18**	**19**	**185**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	6	6	4	6	4	4	4	47
5	12	11	12	10	12	10	10	10	12	12	111
6	0	0	0	0	0	0	0	0	0	0	0
7	18	17	18	14	13	18	13	17	19	19	166
Total	**35**	**32**	**34**	**30**	**31**	**32**	**29**	**31**	**35**	**35**	**324**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	3	2	2	1	1	1	1	3	3	1	18
5	0	0	0	0	1	0	0	0	0	0	1
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	**3**	**2**	**2**	**1**	**2**	**1**	**1**	**3**	**3**	**1**	**19**

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: 305

Test dates: December 03-10, 2024

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	35	32	33	32	34	31	30	32	34	100	32.4	4.9	Not applicable
600	33	32	32	32	36	29	34	34	34	30	100	32.6	6.3	-0.6
800	35	32	34	30	31	32	29	31	35	35	100	32.4	6.9	0.0
1000	33	32	31	28	33	29	30	31	31	29	100	30.7	5.5	5.2
1200	18	19	19	19	21	18	17	17	18	19	100	18.5	6.4	42.9
1400	3	2	2	1	2	1	1	3	3	1	100	1.9	46.1	94.1

Dunnett's MSD value: 1.708

PMSD: 5.3

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	12/3/2024	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	12/10/2024	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	35.000	32.000	33.000	32.000	34.000	31.000	30.000	32.000	34.000
600	33.000	32.000	32.000	32.000	36.000	29.000	34.000	34.000	34.000	30.000
800	35.000	32.000	34.000	30.000	31.000	32.000	29.000	31.000	35.000	35.000
1000	33.000	32.000	31.000	28.000	33.000	29.000	30.000	31.000	31.000	29.000
1200	18.000	19.000	19.000	19.000	21.000	18.000	17.000	17.000	18.000	19.000
1400	3.000	2.000	2.000	1.000	2.000	1.000	1.000	3.000	3.000	1.000

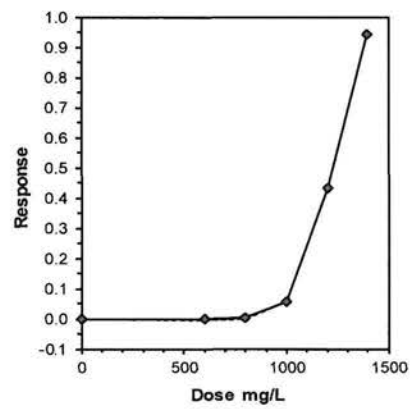
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	32.400	1.0000	32.400	30.000	35.000	4.869	10				32.500	1.0000
600	32.600	1.0062	32.600	29.000	36.000	6.336	10	-0.268	2.287	1.708	32.500	1.0000
800	32.400	1.0000	32.400	29.000	35.000	6.855	10	0.000	2.287	1.708	32.400	0.9969
1000	30.700	0.9475	30.700	28.000	33.000	5.547	10	2.275	2.287	1.708	30.700	0.9446
*1200	18.500	0.5710	18.500	17.000	21.000	6.370	10	18.605	2.287	1.708	18.500	0.5692
*1400	1.900	0.0586	1.900	1.000	3.000	46.084	10	40.825	2.287	1.708	1.900	0.0585

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.77527	1.035	-0.0253	-0.3895
Bartlett's Test indicates equal variances (p = 0.10)	9.24533	15.0863		

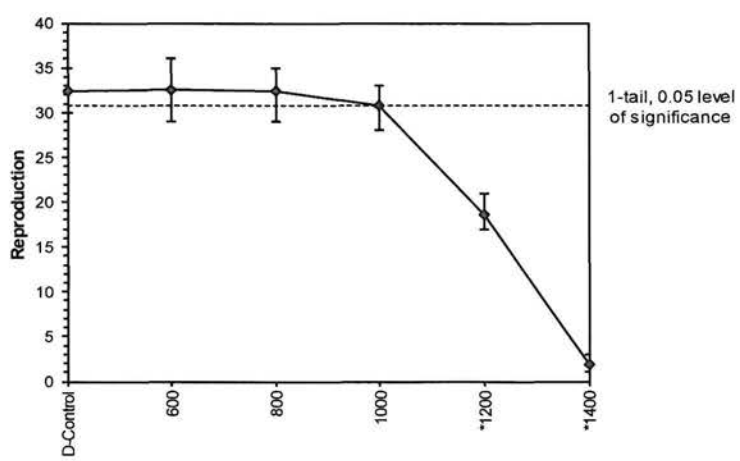
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.70835	0.05273	1550.51	2.79074	4.2E-45	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	979.412	61.43	769.9	1010.97	-1.4988
IC10	1023.77	8.87605	1003.85	1035.95	-0.4085
IC15	1050.41	7.66216	1032.59	1062.18	-0.0646
IC20	1077.05	6.90067	1061.81	1087.95	-0.0974
IC25	1103.69	6.32916	1089.02	1113.2	-0.1372
IC40	1183.61	6.17247	1169.13	1191.59	-0.1685
IC50	1227.11	4.26501	1217.08	1232.55	-0.3419



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 305**

Daily Chemistry:

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

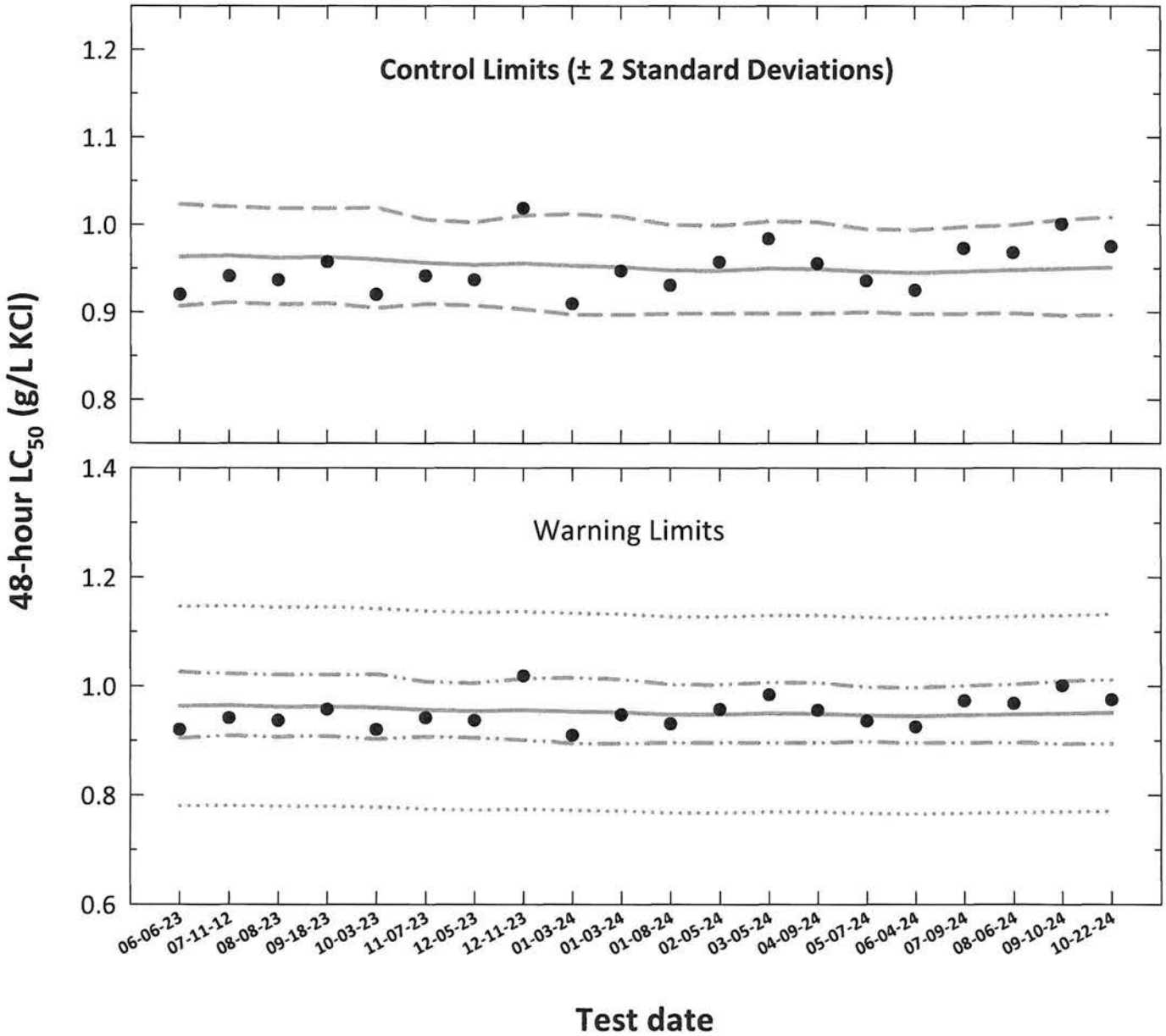
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	K	EM	EM	EM	EM	EM
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.86	7.39	7.47	7.34	7.35	7.50
	Dissolved oxygen (mg/L)	7.6	7.8	7.9	7.6	7.6	7.8
	Conductivity (µmhos/cm)	292		318		317	
	Alkalinity (mg $CaCO_3$/L)	59				57	
	Hardness (mg $CaCO_3$/L)	88				88	
	Temperature (°C)	24.9	25.2	24.8	25.0	24.8	25.1
600 mg NaCl/L	pH (S.U.)	7.87	7.48	7.54	7.41	7.46	7.59
	Dissolved oxygen (mg/L)	7.6	7.8	7.9	7.7	7.6	7.7
	Conductivity (µmhos/cm)	1340		1440		1420	
	Temperature (°C)	25.0	25.2	24.9	25.0	24.9	24.9
800 mg NaCl/L	pH (S.U.)	7.87	7.54	7.55	7.42	7.48	7.60
	Dissolved oxygen (mg/L)	7.6	7.7	7.9	7.7	7.6	7.8
	Conductivity (µmhos/cm)	1710		1850		1830	
	Temperature (°C)	25.0	25.0	24.9	24.9	24.9	24.9
1000 mg NaCl/L	pH (S.U.)	7.87	7.53	7.56	7.43	7.49	7.61
	Dissolved oxygen (mg/L)	7.6	7.8	7.9	7.7	7.6	7.8
	Conductivity (µmhos/cm)	2040		2200 ~~2020~~		2180	
	Temperature (°C)	25.1	24.9	25.0	24.9	25.0	25.2
1200 mg NaCl/L	pH (S.U.)	7.89	7.53	7.57	7.44	7.50	7.62
	Dissolved oxygen (mg/L)	7.7	7.8	7.8	7.7	7.6	7.8
	Conductivity (µmhos/cm)	2390		2580		2540	
	Temperature (°C)	24.9	24.9	24.9	25.1	25.0	25.2
1400 mg NaCl/L	pH (S.U.)	7.89	7.53	7.58	7.47	7.51	7.63
	Dissolved oxygen (mg/L)	7.8	7.8	7.8	7.7	7.6	7.8
	Conductivity (µmhos/cm)	2790		3030		2930	
	Temperature (°C)	24.9	25.1	24.9	24.9	25.0	25.0
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 305**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	EM	N	N	N	N	EM	EM	EM
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.51	7.99	7.81	7.65	7.86	7.44	7.27	7.52
	Dissolved oxygen (mg/L)	7.6	7.9	7.7	7.8	7.9	7.9	7.7	7.8
	Conductivity (µmhos/cm)	315		300		298		310	
	Alkalinity (mg $CaCO_3$/L)			59					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.8	25.2	24.8	25.2	24.8	25.1	24.9	25.3
600 mg NaCl/L	pH (S.U.)	7.65	8.04	7.99	7.67	7.61	7.55	7.52	7.63
	Dissolved oxygen (mg/L)	7.7	7.9	7.8	7.8	8.0	7.9	7.8	7.9
	Conductivity (µmhos/cm)	1430		1370		1350		1430	
	Temperature (°C)	25.0	25.0	24.9	25.0	24.9	25.2	25.0	25.2
800 mg NaCl/L	pH (S.U.)	7.68	8.03	7.97	7.67	7.62	7.57	7.57	7.64
	Dissolved oxygen (mg/L)	7.7	7.9	7.8	7.9	8.0	7.9	7.8	7.9
	Conductivity (µmhos/cm)	1820		1720		1710		1830	
	Temperature (°C)	25.0	25.0	24.9	25.0	25.0	25.2	25.0	25.2
1000 mg NaCl/L	pH (S.U.)	7.70	8.01	7.96	7.66	7.63	7.59	7.60	7.65
	Dissolved oxygen (mg/L)	7.7	7.9	7.9	7.9	8.0	7.8	7.9	7.9
	Conductivity (µmhos/cm)	2170		2060		2040		2170	
	Temperature (°C)	24.8	25.0	24.9	25.0	25.0	25.0	25.0	25.1
1200 mg NaCl/L	pH (S.U.)	7.71	7.98	7.94	7.65	7.63	7.61	7.61	7.65
	Dissolved oxygen (mg/L)	7.7	8.0	7.9	7.9	7.9	7.8	7.9	7.9
	Conductivity (µmhos/cm)	2560		2440		2400		2560	
	Temperature (°C)	24.9	25.1	24.9	24.9	24.9	25.0	25.0	25.1
1400 mg NaCl/L	pH (S.U.)	7.71	7.96	7.93	7.63	7.63	7.61	7.61	7.66
	Dissolved oxygen (mg/L)	7.7	8.0	8.0	7.9	7.9	7.9	7.9	7.9
	Conductivity (µmhos/cm)	2950		2830		2810		2940	
	Temperature (°C)	24.9	24.9	24.9	24.9	25.0	25.0	25.0	25.0
		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	06-06-23	**0.9201**	-0.0362	-0.0162	0.0131	0.9633	0.9068	1.0233	0.9046	1.0256	0.7803	1.1463
2	07-11-12	**0.9413**	-0.0263	-0.0156	0.0123	0.9646	0.9116	1.0208	0.9096	1.0229	0.7814	1.1479
3	08-08-23	**0.9366**	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208	0.7793	1.1449
4	09-18-23	**0.9572**	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
5	10-03-23	**0.9201**	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
6	11-07-23	**0.9413**	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378
7	12-05-23	**0.9368**	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052	0.7728	1.1353
8	12-11-23	**1.0186**	0.0080	-0.0197	0.0122	0.9556	0.9035	1.0107	0.9011	1.0133	0.7740	1.1372
9	01-03-24	**0.9095**	-0.0412	-0.0208	0.0131	0.9532	0.8974	1.0124	0.8947	1.0153	0.7721	1.1343
10	01-03-24	**0.9470**	-0.0236	-0.0216	0.0128	0.9516	0.8971	1.0093	0.8943	1.0123	0.7708	1.1323
11	01-08-24	**0.9307**	-0.0312	-0.0233	0.0116	0.9478	0.8985	0.9999	0.8958	1.0027	0.7678	1.1279
12	02-05-24	**0.9572**	-0.0190	-0.0234	0.0115	0.9475	0.8986	0.9992	0.8958	1.0020	0.7675	1.1276
13	03-05-24	**0.9839**	-0.0070	-0.0223	0.0120	0.9499	0.8988	1.0039	0.8961	1.0067	0.7694	1.1304
14	04-09-24	**0.9556**	-0.0197	-0.0225	0.0119	0.9495	0.8987	1.0031	0.8961	1.0060	0.7691	1.1299
15	05-07-24	**0.9358**	-0.0288	-0.0239	0.0109	0.9465	0.9002	0.9952	0.8976	0.9979	0.7667	1.1263
16	06-04-24	**0.9251**	-0.0338	-0.0246	0.0110	0.9449	0.8980	0.9942	0.8953	0.9970	0.7653	1.1244
17	07-09-24	**0.9729**	-0.0119	-0.0238	0.0114	0.9467	0.8985	0.9975	0.8958	1.0004	0.7668	1.1266
18	08-06-24	**0.9680**	-0.0141	-0.0232	0.0115	0.9481	0.8991	0.9998	0.8964	1.0026	0.7679	1.1282
19	09-10-24	**1.0007**	0.0003	-0.0224	0.0125	0.9496	0.8964	1.0060	0.8936	1.0090	0.7692	1.1301
20	10-22-24	**0.9751**	-0.0109	-0.0217	0.0128	0.9513	0.8970	1.0088	0.8942	1.0118	0.7705	1.1320

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 # 169

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

Stock solution INSS #: 2319

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	K	BM	BM
Control, MHSW	pH (S.U.)	7.94	7.63	7.22
	Dissolved oxygen (mg/L)	7.8	7.7	7.3
	Conductivity (µmhos/cm)	307		
	Alkalinity (mg/L $CaCO_3$)	58		
	Hardness (mg/L $CaCO_3$)	84		
	Temperature (°C)	24.7	24.6	24.7
500 mg/L	pH (S.U.)	7.99	7.69	7.45
	Dissolved oxygen (mg/L)	7.6	7.6	7.4
	Conductivity (µmhos/cm)	1160		
	Temperature (°C)	24.8	24.7	24.5
750 mg/L	pH (S.U.)	7.96	7.72	7.50
	Dissolved oxygen (mg/L)	7.6	7.6	7.4
	Conductivity (µmhos/cm)	1560		
	Temperature (°C)	24.8	24.7	24.8
1000 mg/L	pH (S.U.)	7.95	7.70	7.52
	Dissolved oxygen (mg/L)	7.6	7.6	7.5
	Conductivity (µmhos/cm)	1920		
	Temperature (°C)	24.9	24.5	24.6
1250 mg/L	pH (S.U.)	7.95	7.70	7.56
	Dissolved oxygen (mg/L)	7.7	7.5	7.4
	Conductivity (µmhos/cm)	2320		
	Temperature (°C)	24.9	24.5	24.6
1500 mg/L	pH (S.U.)	7.96	7.70	
	Dissolved oxygen (mg/L)	7.8	7.5	
	Conductivity (µmhos/cm)	2760		10-23-24
	Temperature (°C)	24.8	24.8	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial numb
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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 applicab
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1306646

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 # 169

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	10-22-24	* 0500	JH	0700	JH	1D	WHITE	10-20-24
24	10-23-24			0655	JH			
48 Termination	10-24-24			0658	JH			

*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:	10-14-24
Age (1 to 14 days old):	2-3 DAYS
Hatch date and times:	10-19-24 1500 TO 10-20-24 0450
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.02
	Temperature (°C): 24.2°C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	5 sd	5 sd	2 sd	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	9 1d	10	4 1d	4 1d	1 1d	1	0	0
Mean Survival	100%		100%		95%		40%		10%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	901.8
Upper 95% confidence limit (mg KCl/L)	1044.6
48-hour LC_{50} (mg KCl/L)	975.1

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:	10/22/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	10/24/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

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

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	13.965	2.850	0.1279	10	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	22.716	2.850	0.1279	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

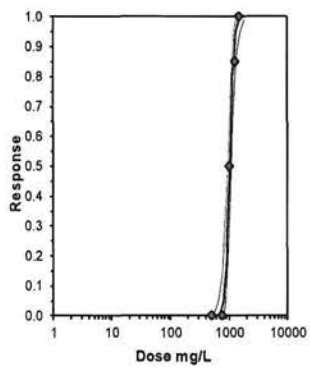
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.05495	0.05636	0.44492	0.00201	8.3E-06	4, 5
Treatments vs D-Control										

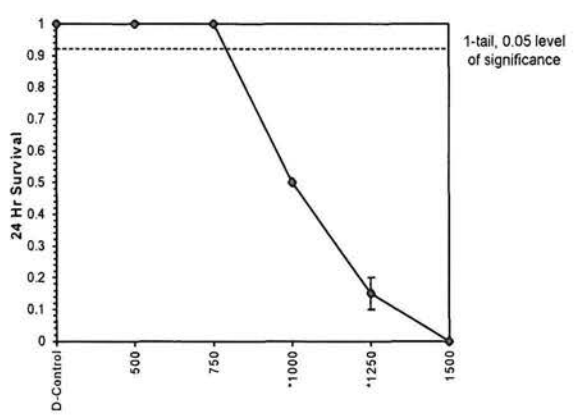
Maximum Likelihood-Probit

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

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



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	10/22/2024	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	10/24/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	1.0000
1000	0.4000	0.4000
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	0.4000	0.4000	0.6847	0.6847	0.6847	0.000	2	14.112	2.850	0.1469	12	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

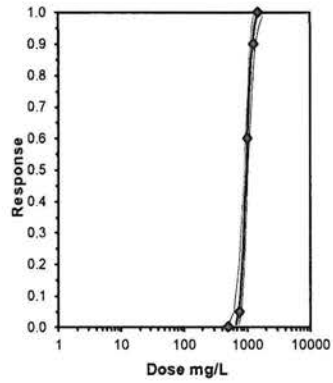
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

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	0.5015	0.00266	1.2E-05	4, 5
Treatments vs D-Control										

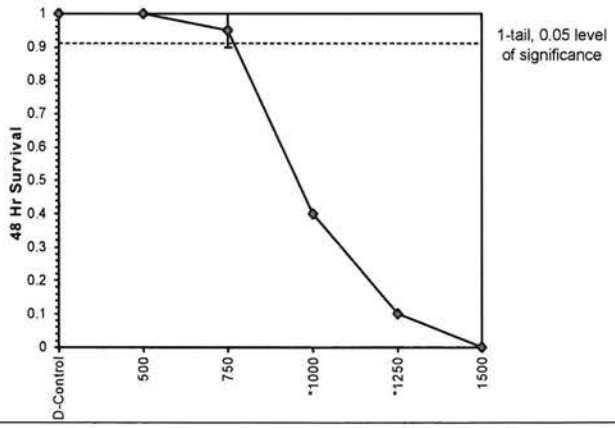
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.5835	2.49087	8.70135	18.4655	0	0.53397	7.81472	0.91137	2.98906	0.07362	3
Intercept	-35.602	7.48214	-50.267	-20.937							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	657.356	510.311	746.281
EC05	3.355	737.856	608.289	816.309
EC10	3.718	784.725	667.059	857.486
EC15	3.964	818.018	709.227	887.259
EC20	4.158	845.483	744.062	912.359
EC25	4.326	869.779	774.744	935.136
EC40	4.747	934.142	854.078	999.407
EC50	5.000	975.134	901.835	1044.6
EC60	5.253	1017.92	948.374	1096.31
EC75	5.674	1093.25	1021.68	1198.96
EC80	5.842	1124.67	1049.51	1245.63
EC85	6.036	1162.43	1081.38	1304.18
EC90	6.282	1211.74	1120.99	1384.07
EC95	6.645	1288.71	1179.66	1515.07
EC99	7.326	1446.53	1292.63	1802.77

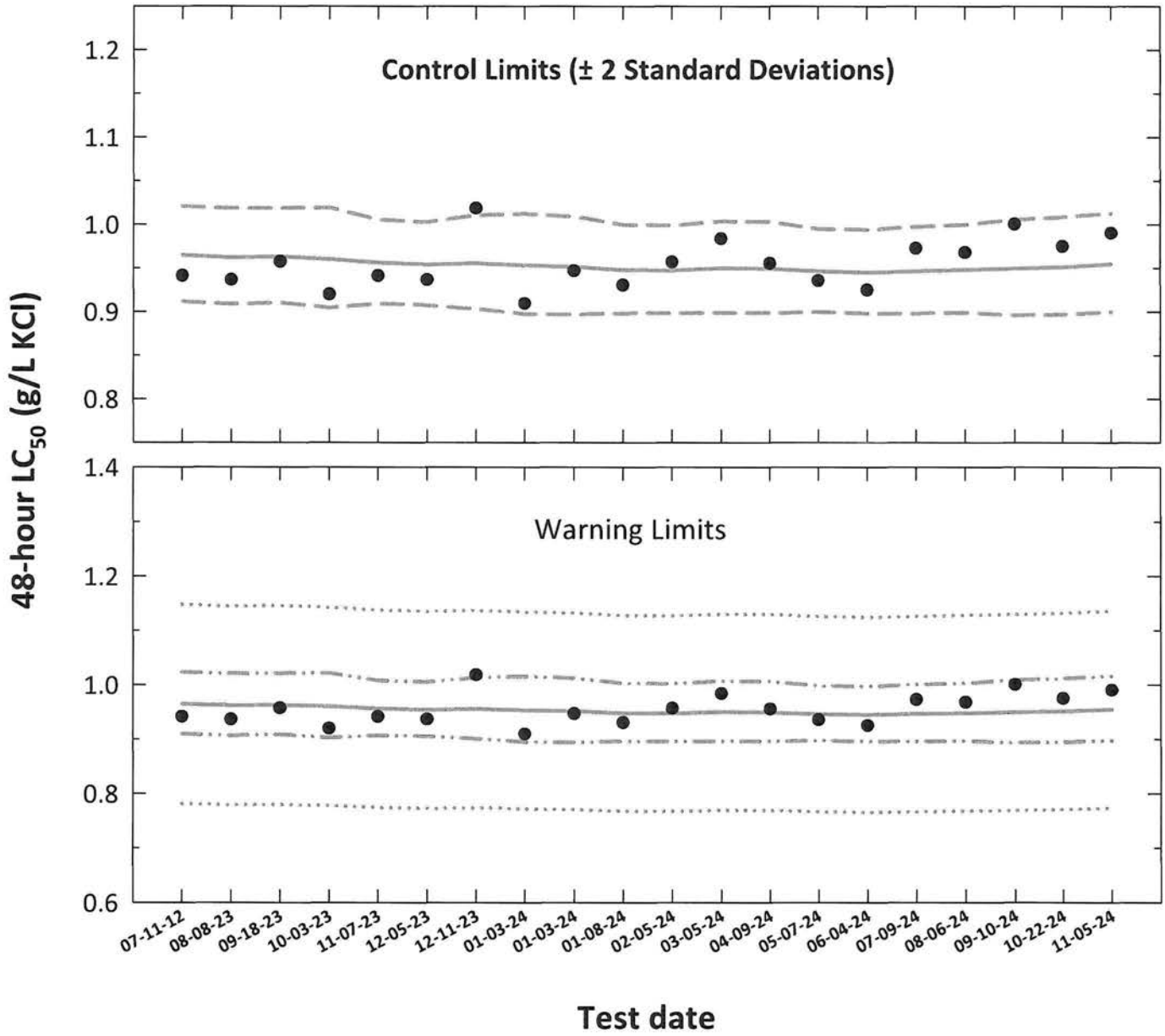


Dose-Response Plot



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 $LC_{50} \pm S_{A.75}$ 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	07-11-12	0.9413	-0.0263	-0.0156	0.0123	0.9646	0.9116	1.0208	0.9096	1.0229	0.7814	1.1479
2	08-08-23	0.9366	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208	0.7793	1.1449
3	09-18-23	0.9572	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
4	10-03-23	0.9201	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
5	11-07-23	0.9413	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378
6	12-05-23	0.9368	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052	0.7728	1.1353
7	12-11-23	1.0186	0.0080	-0.0197	0.0122	0.9556	0.9035	1.0107	0.9011	1.0133	0.7740	1.1372
8	01-03-24	0.9095	-0.0412	-0.0208	0.0131	0.9532	0.8974	1.0124	0.8947	1.0153	0.7721	1.1343
9	01-03-24	0.9470	-0.0236	-0.0216	0.0128	0.9516	0.8971	1.0093	0.8943	1.0123	0.7708	1.1323
10	01-08-24	0.9307	-0.0312	-0.0233	0.0116	0.9478	0.8985	0.9999	0.8958	1.0027	0.7678	1.1279
11	02-05-24	0.9572	-0.0190	-0.0234	0.0115	0.9475	0.8986	0.9992	0.8958	1.0020	0.7675	1.1276
12	03-05-24	0.9839	-0.0070	-0.0223	0.0120	0.9499	0.8988	1.0039	0.8961	1.0067	0.7694	1.1304
13	04-09-24	0.9556	-0.0197	-0.0225	0.0119	0.9495	0.8987	1.0031	0.8961	1.0060	0.7691	1.1299
14	05-07-24	0.9358	-0.0288	-0.0239	0.0109	0.9465	0.9002	0.9952	0.8976	0.9979	0.7667	1.1263
15	06-04-24	0.9251	-0.0338	-0.0246	0.0110	0.9449	0.8980	0.9942	0.8953	0.9970	0.7653	1.1244
16	07-09-24	0.9729	-0.0119	-0.0238	0.0114	0.9467	0.8985	0.9975	0.8958	1.0004	0.7668	1.1266
17	08-06-24	0.9680	-0.0141	-0.0232	0.0115	0.9481	0.8991	0.9998	0.8964	1.0026	0.7679	1.1282
18	09-10-24	1.0007	0.0003	-0.0224	0.0125	0.9496	0.8964	1.0060	0.8936	1.0090	0.7692	1.1301
19	10-22-24	0.9751	-0.0109	-0.0217	0.0128	0.9513	0.8970	1.0088	0.8942	1.0118	0.7705	1.1320
20	11-05-24	0.9904	-0.0042	-0.0201	0.0128	0.9548	0.9000	1.0130	0.8974	1.0157	0.7734	1.1362

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC_{50} values.

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

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

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.75}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*
EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 170

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 #: 2319

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	N	N	EM
Control, MHSW	pH (S.U.)	7.71	7.64	7.68
	Dissolved oxygen (mg/L)	7.7	7.7	7.7
	Conductivity (µmhos/cm)	298		
	Alkalinity (mg/L $CaCO_3$)	61		
	Hardness (mg/L $CaCO_3$)	92		
	Temperature (°C)	24.8	25.0	24.9
500 mg/L	pH (S.U.)	7.96	7.64	7.71
	Dissolved oxygen (mg/L)	7.8	7.7	7.7
	Conductivity (µmhos/cm)	1170		
	Temperature (°C)	24.9	24.8	25.0
750 mg/L	pH (S.U.)	7.96	7.68	7.72
	Dissolved oxygen (mg/L)	7.8	7.6	7.7
	Conductivity (µmhos/cm)	1540		
	Temperature (°C)	24.9	24.8	24.7
1000 mg/L	pH (S.U.)	7.95	7.67	7.73
	Dissolved oxygen (mg/L)	7.8	7.6	7.7
	Conductivity (µmhos/cm)	1950		
	Temperature (°C)	24.7	24.9	24.7
1250 mg/L	pH (S.U.)	7.96	7.66	7.71
	Dissolved oxygen (mg/L)	7.8	7.7	7.6
	Conductivity (µmhos/cm)	2330		
	Temperature (°C)	24.9	24.9	25.0
1500 mg/L	pH (S.U.)	7.96	7.66	
	Dissolved oxygen (mg/L)	7.8	7.7	11.06.24
	Conductivity (µmhos/cm)	2790		
	Temperature (°C)	24.9	25.1	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by th analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identifie 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 G-2021	YSI Model 52CE	08A100271
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	13066468

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 # 170

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	11-05-24	* 0500	JI	0700	JI	6D	LIGHT BLUE	10-30-24A
24	11-06-24			0650	JI			
48 Termination	11-07-24			0650	JI			

*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:	10-24-24
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	10-29-24 1504 TO 10-30-24 0530
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.96
	Temperature (°C): 24.4

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	8 2d	8 2d	2 8d	3 7d	0 10d	0 10d
48 Termination	10	10	10	10	9 1d	9 1d	5 3d	5 3d	2 8	1 2d	0	0
Mean Survival	100%		100%		90%		50%		15%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	909.9
Upper 95% confidence limit (mg KCl/L)	1066.7
48-hour LC_{50} (mg KCl/L)	990.4

Comments:

Test Reviewed by: JI

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	11/5/2024	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	11/7/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	1.0000	1.0000
1000	0.8000	0.8000
1250	0.2000	0.3000
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	0.1045	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1045	0	20
*1000	0.8000	0.8000	1.1071	1.1071	1.1071	0.000	2	8.312	2.850	0.1045	4	20
*1250	0.2500	0.2500	0.5216	0.4636	0.5796	15.723	2	24.274	2.850	0.1045	15	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

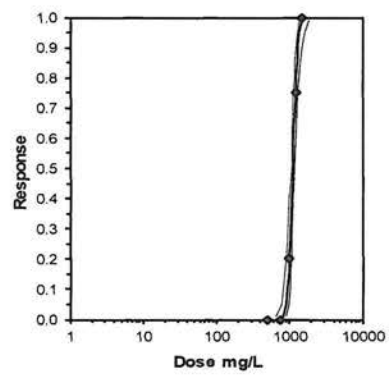
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.04275	0.04384	0.29999	0.00135	8.1E-06	4, 5
Treatments vs D-Control										

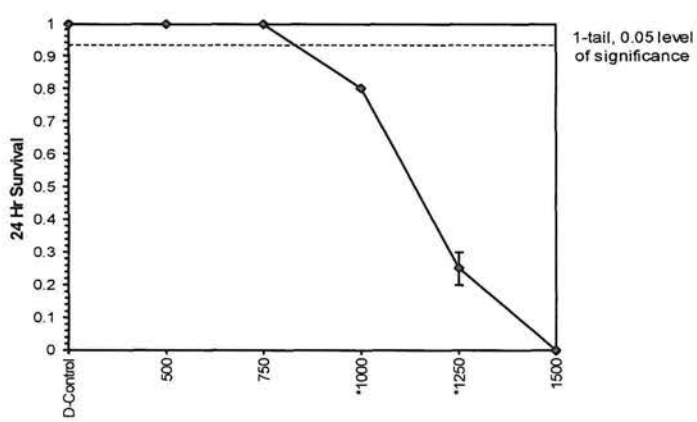
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.7389	3.5689	10.7439	24.734	0	0.53223	7.81472	0.91176	3.05161	0.05637	4
Intercept	-49.132	10.9202	-70.536	-27.729							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	832.661	668.515	922.003
EC05	3.355	909.675	770.606	986.269
EC10	3.718	953.602	830.247	1023.58
EC15	3.964	984.43	872.353	1050.42
EC20	4.158	1009.64	906.705	1073
EC25	4.326	1031.78	936.632	1093.47
EC40	4.747	1089.76	1012.41	1151.45
EC50	5.000	1126.19	1056.73	1192.51
EC60	5.253	1163.84	1098.83	1239.71
EC75	5.674	1229.24	1163.06	1333.13
EC80	5.842	1256.19	1186.9	1375.22
EC85	6.036	1288.37	1213.91	1427.61
EC90	6.282	1330.02	1247.14	1498.32
EC95	6.645	1394.24	1295.72	1612.55
EC99	7.326	1523.2	1387.47	1856.88



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:	11/5/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	11/7/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

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

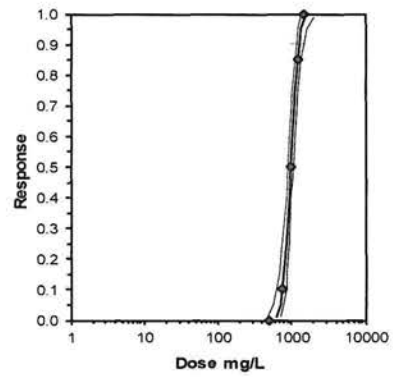
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1279	0	20
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	3.632	2.850	0.1279	2	20
*1000	0.5000	0.5000	0.7854	0.7854	0.7854	0.000	2	13.965	2.850	0.1279	10	20
*1250	0.1500	0.1500	0.3927	0.3218	0.4636	25.550	2	22.716	2.850	0.1279	17	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

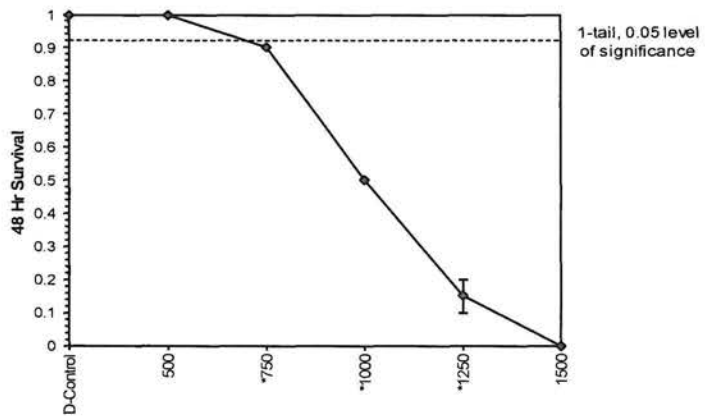
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.05495	0.05636	0.40189	0.00201	1.1E-05	4, 5
Treatments vs D-Control										

					Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.4402	2.01873	7.48343	15.3969	0	0.63348	7.81472	0.88873	2.9958	0.08741	4
Intercept	-29.272	6.08432	-41.198	-17.347							
TSCR											

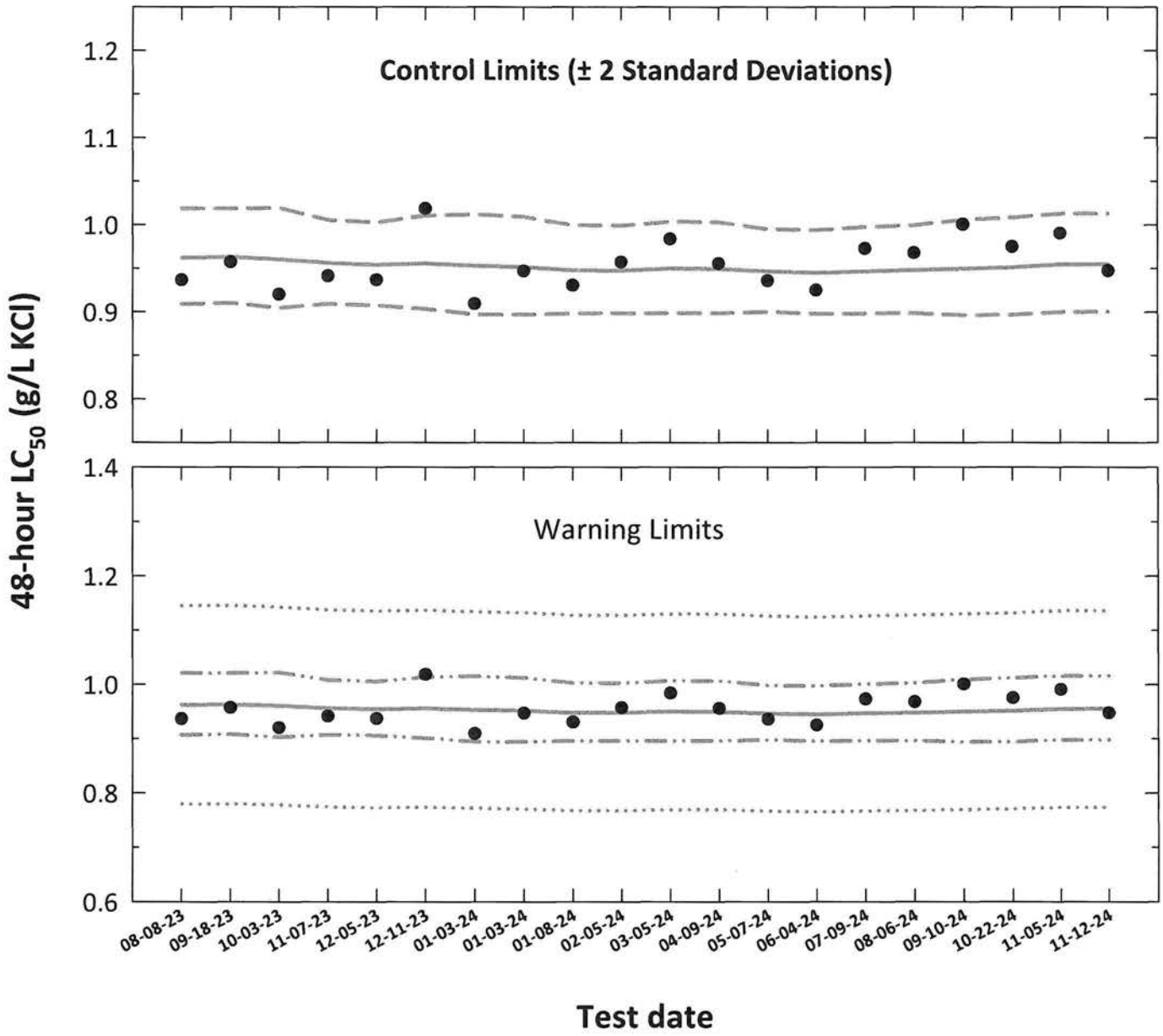
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	620.087	467.401	716.82
EC05	3.355	711.251	573.63	797.621
EC10	3.718	765.208	638.861	845.602
EC15	3.964	803.907	686.331	880.468
EC20	4.158	836.054	725.958	909.957
EC25	4.326	864.657	761.169	936.784
EC40	4.747	941.145	853.511	1012.84
EC50	5.000	990.38	909.943	1066.71
EC60	5.253	1042.19	965.457	1128.85
EC75	5.674	1134.38	1053.83	1253.79
EC80	5.842	1173.19	1087.71	1311.21
EC85	6.036	1220.11	1126.74	1383.72
EC90	6.282	1281.81	1175.65	1483.43
EC95	6.645	1379.05	1248.87	1648.82
EC99	7.326	1581.8	1392.33	2019.65



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**08-08-23**	**0.9366**	-0.0284	-0.0168	0.0124	0.9621	0.9088	1.0185	0.9067	1.0208	0.7793	1.1449
2	**09-18-23**	**0.9572**	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
3	**10-03-23**	**0.9201**	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
4	**11-07-23**	**0.9413**	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378
5	**12-05-23**	**0.9368**	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052	0.7728	1.1353
6	**12-11-23**	**1.0186**	0.0080	-0.0197	0.0122	0.9556	0.9035	1.0107	0.9011	1.0133	0.7740	1.1372
7	**01-03-24**	**0.9095**	-0.0412	-0.0208	0.0131	0.9532	0.8974	1.0124	0.8947	1.0153	0.7721	1.1343
8	**01-03-24**	**0.9470**	-0.0236	-0.0216	0.0128	0.9516	0.8971	1.0093	0.8943	1.0123	0.7708	1.1323
9	**01-08-24**	**0.9307**	-0.0312	-0.0233	0.0116	0.9478	0.8985	0.9999	0.8958	1.0027	0.7678	1.1279
10	**02-05-24**	**0.9572**	-0.0190	-0.0234	0.0115	0.9475	0.8986	0.9992	0.8958	1.0020	0.7675	1.1276
11	**03-05-24**	**0.9839**	-0.0070	-0.0223	0.0120	0.9499	0.8988	1.0039	0.8961	1.0067	0.7694	1.1304
12	**04-09-24**	**0.9556**	-0.0197	-0.0225	0.0119	0.9495	0.8987	1.0031	0.8961	1.0060	0.7691	1.1299
13	**05-07-24**	**0.9358**	-0.0288	-0.0239	0.0109	0.9465	0.9002	0.9952	0.8976	0.9979	0.7667	1.1263
14	**06-04-24**	**0.9251**	-0.0338	-0.0246	0.0110	0.9449	0.8980	0.9942	0.8953	0.9970	0.7653	1.1244
15	**07-09-24**	**0.9729**	-0.0119	-0.0238	0.0114	0.9467	0.8985	0.9975	0.8958	1.0004	0.7668	1.1266
16	**08-06-24**	**0.9680**	-0.0141	-0.0232	0.0115	0.9481	0.8991	0.9998	0.8964	1.0026	0.7679	1.1282
17	**09-10-24**	**1.0007**	0.0003	-0.0224	0.0125	0.9496	0.8964	1.0060	0.8936	1.0090	0.7692	1.1301
18	**10-22-24**	**0.9751**	-0.0109	-0.0217	0.0128	0.9513	0.8970	1.0088	0.8942	1.0118	0.7705	1.1320
19	**11-05-24**	**0.9904**	-0.0042	-0.0201	0.0128	0.9548	0.9000	1.0130	0.8974	1.0157	0.7734	1.1362
20	**11-12-24**	**0.9473**	-0.0235	-0.0200	0.0128	0.9551	0.9005	1.0130	0.8979	1.0157	0.7736	1.1366

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC_{50} values.

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

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

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.75}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*
EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 171

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 #: 2334

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	NJP	BM	A
Control, MHSW	pH (S.U.)	7.66	7.41	7.56
	Dissolved oxygen (mg/L)	7.9	7.7	7.5
	Conductivity (µmhos/cm)	302		
	Alkalinity (mg/L $CaCO_3$)	62		
	Hardness (mg/L $CaCO_3$)	90		
	Temperature (°C)	24.4	24.8	25.2
500 mg/L	pH (S.U.)	7.91	7.54	7.49
	Dissolved oxygen (mg/L)	7.9	7.6	7.1
	Conductivity (µmhos/cm)	1150		
	Temperature (°C)	24.3	24.9	24.7
750 mg/L	pH (S.U.)	7.92	7.56	7.49
	Dissolved oxygen (mg/L)	7.9	7.6	7.0
	Conductivity (µmhos/cm)	1560		
	Temperature (°C)	24.6	24.9	24.8
1000 mg/L	pH (S.U.)	7.92	7.57	7.49
	Dissolved oxygen (mg/L)	7.9	7.7	7.0
	Conductivity (µmhos/cm)	2000		
	Temperature (°C)	24.3	24.7	25.0
1250 mg/L	pH (S.U.)	7.91	7.48	7.45
	Dissolved oxygen (mg/L)	7.9	7.7	7.0
	Conductivity (µmhos/cm)	2410		
	Temperature (°C)	24.4	24.7	24.7
1500 mg/L	pH (S.U.)	7.92	7.51	7.42
	Dissolved oxygen (mg/L)	7.9	7.7	7.0
	Conductivity (µmhos/cm)	2910		
	Temperature (°C)	24.1	24.6	24.7

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by th analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identifie 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 G-2021	YSI Model 52CE	08A100271
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	1118984

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 # 171

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	11-12-24	*0628	JP	1144	JP	7B	yellow	11-08-24
24	11-13-24			1146	JP			
48 Termination	11-14-24			1141	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:	10-30-24
Age (1 to 14 days old):	7 to 8 days old
Hatch date and times:	11-04-24 1500 to 11-05-24 0405
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.86
	Temperature (°C): 24.1 °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	9 1D	9 12	3 7D	5 5D	0 10D	0 10D	1 4D	0 10D
48 Termination	10	10	10	10	9	9	3	5	0	0	1	0
Mean Survival	100 %		100 %		90 %		40 %		0 %		5 %	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	869.5
Upper 95% confidence limit (mg KCl/L)	1020.3
48-hour LC_{50} (mg KCl/L)	947.3

Comments:

Test Reviewed by: AL

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 and 48 Hr Survival

Start Date:	11/12/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	11/14/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	0.9000
1000	0.3000	0.5000
1250	0.0000	0.0000
1500	0.1000	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	2.830	0.2144	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.151	2.830	0.2144	2	20
*1000	0.4000	0.4000	0.6825	0.5796	0.7854	21.317	2	9.628	2.830	0.2144	12	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	16.540	2.830	0.2144	20	20
*1500	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	15.464	2.830	0.2144	19	20

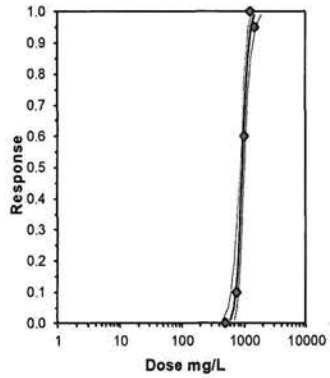
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.10794	0.11071	0.66723	0.00574	6.9E-06	5, 6
Treatments vs D-Control										

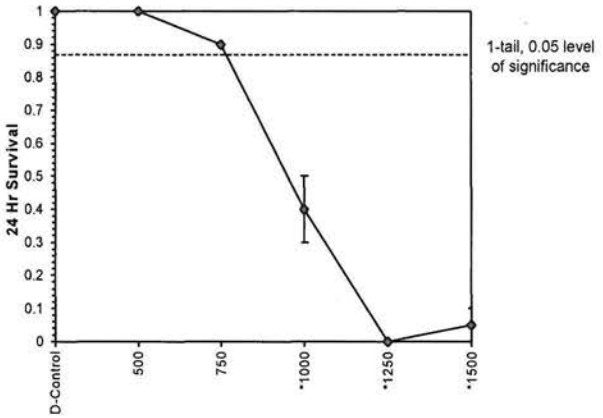
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.8068	2.11279	7.6657	15.9478	0	5.39122	7.81472	0.14529	2.97649	0.0847	4
Intercept	-30.143	6.32896	-42.547	-17.738							
TSCR											

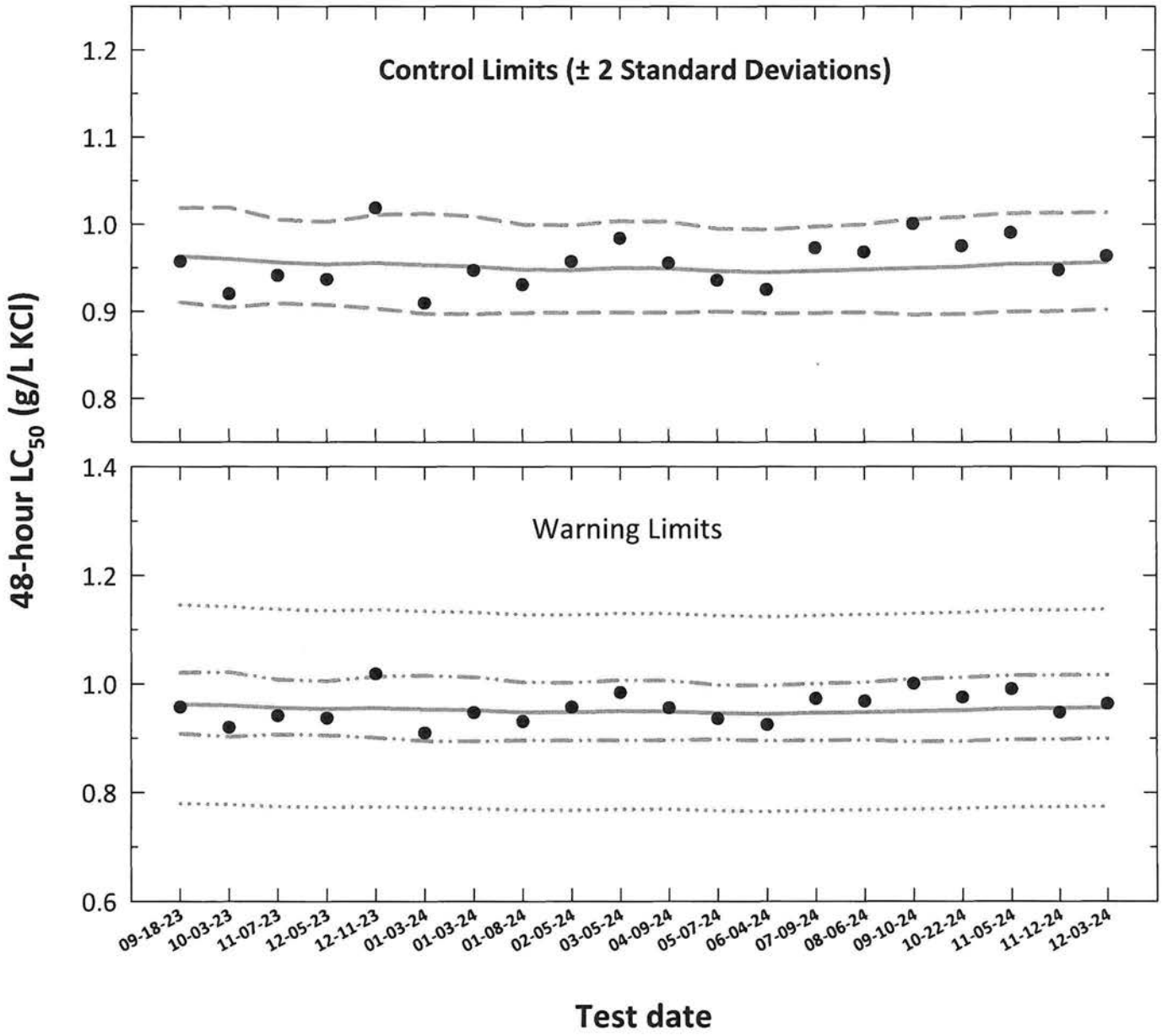
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	601.801	453.912	694.536
EC05	3.355	687.342	554.3	770.117
EC10	3.718	737.809	615.701	814.902
EC15	3.964	773.935	660.284	847.404
EC20	4.158	803.905	697.442	874.868
EC25	4.326	830.539	730.42	899.829
EC40	4.747	901.633	816.778	970.453
EC50	5.000	947.3	869.51	1020.33
EC60	5.253	995.28	921.375	1077.74
EC75	5.674	1080.48	1003.83	1192.98
EC80	5.842	1116.27	1035.36	1245.91
EC85	6.036	1159.5	1071.6	1312.73
EC90	6.282	1216.27	1116.89	1404.57
EC95	6.645	1305.58	1184.44	1556.74
EC99	7.326	1491.15	1316.13	1896.98



Dose-Response Plot



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



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	**09-18-23**	**0.9572**	-0.0190	-0.0164	0.0122	0.9629	0.9103	1.0185	0.9083	1.0207	0.7800	1.1459
2	**10-03-23**	**0.9201**	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
3	**11-07-23**	**0.9413**	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378
4	**12-05-23**	**0.9368**	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052	0.7728	1.1353
5	**12-11-23**	**1.0186**	0.0080	-0.0197	0.0122	0.9556	0.9035	1.0107	0.9011	1.0133	0.7740	1.1372
6	**01-03-24**	**0.9095**	-0.0412	-0.0208	0.0131	0.9532	0.8974	1.0124	0.8947	1.0153	0.7721	1.1343
7	**01-03-24**	**0.9470**	-0.0236	-0.0216	0.0128	0.9516	0.8971	1.0093	0.8943	1.0123	0.7708	1.1323
8	**01-08-24**	**0.9307**	-0.0312	-0.0233	0.0116	0.9478	0.8985	0.9999	0.8958	1.0027	0.7678	1.1279
9	**02-05-24**	**0.9572**	-0.0190	-0.0234	0.0115	0.9475	0.8986	0.9992	0.8958	1.0020	0.7675	1.1276
10	**03-05-24**	**0.9839**	-0.0070	-0.0223	0.0120	0.9499	0.8988	1.0039	0.8961	1.0067	0.7694	1.1304
11	**04-09-24**	**0.9556**	-0.0197	-0.0225	0.0119	0.9495	0.8987	1.0031	0.8961	1.0060	0.7691	1.1299
12	**05-07-24**	**0.9358**	-0.0288	-0.0239	0.0109	0.9465	0.9002	0.9952	0.8976	0.9979	0.7667	1.1263
13	**06-04-24**	**0.9251**	-0.0338	-0.0246	0.0110	0.9449	0.8980	0.9942	0.8953	0.9970	0.7653	1.1244
14	**07-09-24**	**0.9729**	-0.0119	-0.0238	0.0114	0.9467	0.8985	0.9975	0.8958	1.0004	0.7668	1.1266
15	**08-06-24**	**0.9680**	-0.0141	-0.0232	0.0115	0.9481	0.8991	0.9998	0.8964	1.0026	0.7679	1.1282
16	**09-10-24**	**1.0007**	0.0003	-0.0224	0.0125	0.9496	0.8964	1.0060	0.8936	1.0090	0.7692	1.1301
17	**10-22-24**	**0.9751**	-0.0109	-0.0217	0.0128	0.9513	0.8970	1.0088	0.8942	1.0118	0.7705	1.1320
18	**11-05-24**	**0.9904**	-0.0042	-0.0201	0.0128	0.9548	0.9000	1.0130	0.8974	1.0157	0.7734	1.1362
19	**11-12-24**	**0.9473**	-0.0235	-0.0200	0.0128	0.9551	0.9005	1.0130	0.8979	1.0157	0.7736	1.1366
20	**12-03-24**	**0.9637**	-0.0160	-0.0193	0.0127	0.9565	0.9023	1.0138	0.8998	1.0165	0.7747	1.1382

Note: **48-hour LC_{50}** = 48-hour median lethal concentration. An estimate of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).

CT = Central tendency of the LC_{50} values.

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

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

Warning Limits = Mean logarithmic LC_{50} ± 2CV or $S_{A.75}$ converted to anti-logarithmic values.

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

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

Acute LC_{50} Whole Effluent Toxicity Test, Species: *Pimephales promelas*
EPA-821-R-02-012, Method 2000.0

Pimephales promelas Potassium Chloride Acute Reference Toxicant Test

PpKClAC # 172

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

Stock solution INSS #: 2334

Chemical Analyses:

Concentration	Analyst	Hours 0	24	48
	Analyst	N	EM	EM
Control, MHSW	pH (S.U.)	7.86	7.61	7.34
	Dissolved oxygen (mg/L)	7.4	7.9	7.7
	Conductivity (µmhos/cm)	292		
	Alkalinity (mg/L $CaCO_3$)	59		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	24.9	25.1	24.8
500 mg/L	pH (S.U.)	7.81	7.62	7.41
	Dissolved oxygen (mg/L)	7.8	7.9	7.7
	Conductivity (µmhos/cm)	1120		
	Temperature (°C)	25.0	25.0	25.3
750 mg/L	pH (S.U.)	7.80	7.63	7.41
	Dissolved oxygen (mg/L)	7.8	7.8	7.8
	Conductivity (µmhos/cm)	1520		
	Temperature (°C)	25.0	25.0	25.0
1000 mg/L	pH (S.U.)	7.78	7.60	7.40
	Dissolved oxygen (mg/L)	7.8	7.8	7.7
	Conductivity (µmhos/cm)	1920		
	Temperature (°C)	24.8	25.2	25.0
1250 mg/L	pH (S.U.)	7.78	7.61	7.48
	Dissolved oxygen (mg/L)	7.9	7.9	7.7
	Conductivity (µmhos/cm)	2330		
	Temperature (°C)	24.8	24.9	24.9
1500 mg/L	pH (S.U.)	7.78	7.59	
	Dissolved oxygen (mg/L)	8.0	7.9	
	Conductivity (µmhos/cm)	2700		12.04.24
	Temperature (°C)	25.0	24.9	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial numb
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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 applicab
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	1306461

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 # 172

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	12-03-24	* 0505	JL	0705	JL	7F	GREEN	11-27-24A
24	12-04-24			0655	JL			
48 Termination	12-05-24			0655	JL			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	11-21-24
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	11-26-24 1557 TO 11-27-24 0826
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.68
	Temperature (°C): 24.0°C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	3 7d	5 5d	1 9d	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	9 1d	10	3	5	0 1d	1	0	0
Mean Survival	100%		100%		95%		40%		5%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	894.1
Upper 95% confidence limit (mg KCl/L)	1029.8
48-hour LC_{50} (mg KCl/L)	963.7

Comments:

Test Reviewed by: JL

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	12/3/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	12/5/2024	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	0.3000	0.5000
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	0.1854	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1854	0	20
*1000	0.4000	0.4000	0.6825	0.5796	0.7854	21.317	2	11.212	2.850	0.1854	12	20
*1250	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	2	16.756	2.850	0.1854	18	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

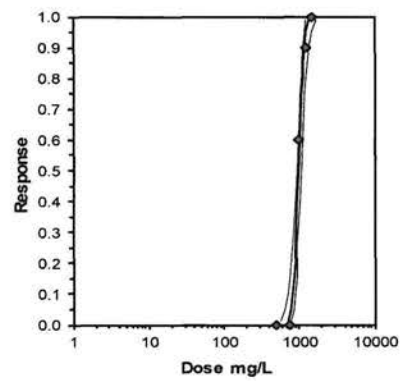
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08888	0.09116	0.52927	0.00423	3.4E-05	4, 5
Treatments vs D-Control										

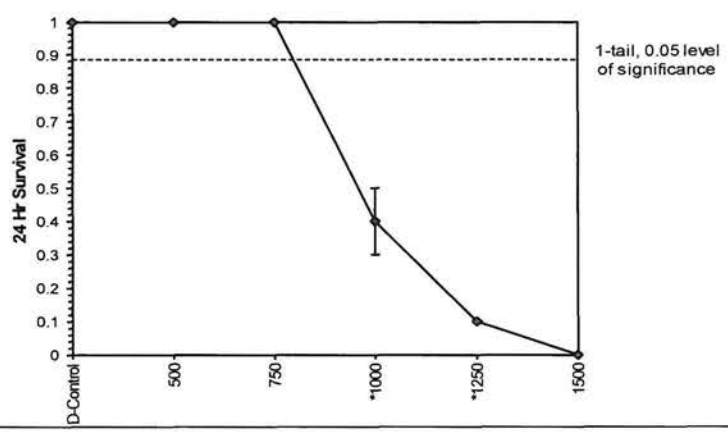
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.9474	3.139	9.79496	22.0998	0	1.83827	7.81472	0.60664	2.99825	0.06271	6
Intercept	-42.814	9.45076	-61.338	-24.291							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	711.826	559.34	797.927
EC05	3.355	785.43	653.679	860.375
EC10	3.718	827.73	709.394	896.799
EC15	3.964	857.55	748.997	923.035
EC20	4.158	882.014	781.486	945.109
EC25	4.326	903.557	809.931	965.117
EC40	4.747	960.204	882.706	1021.56
EC50	5.000	995.979	925.912	1061.3
EC60	5.253	1033.09	967.499	1106.85
EC75	5.674	1097.85	1031.83	1197.24
EC80	5.842	1124.67	1055.88	1238.23
EC85	6.036	1156.75	1083.17	1289.5
EC90	6.282	1198.43	1116.79	1359.14
EC95	6.645	1262.97	1166.02	1472.51
EC99	7.326	1393.56	1259.34	1718.05



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:	12/3/2024	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	12/5/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.9000	1.0000
1000	0.3000	0.5000
1250	0.0000	0.1000
1500	0.0000	0.0000

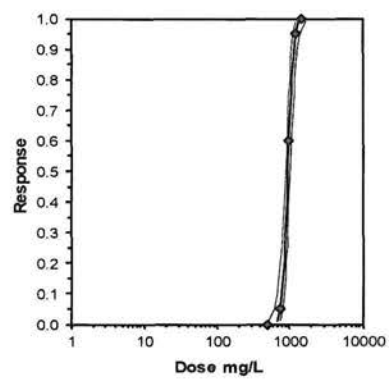
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.2784	0	20
750	0.9500	0.9500	1.3305	1.2490	1.4120	8.661	2	0.834	2.850	0.2784	1	20
*1000	0.4000	0.4000	0.6825	0.5796	0.7854	21.317	2	7.467	2.850	0.2784	12	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	11.993	2.850	0.2784	19	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

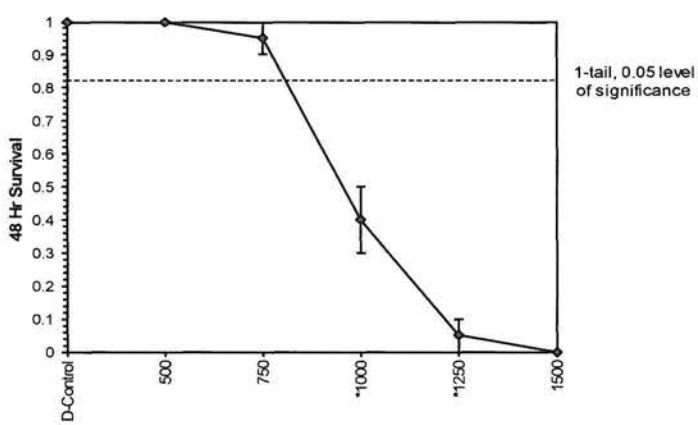
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.15429	0.15825	0.56278	0.00955	2.1E-04	4, 5
Treatments vs D-Control										

					Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.0656	2.87343	9.43368	20.6975	0	0.05472	7.81472	0.99665	2.98396	0.06638	3
Intercept	-39.955	8.61041	-56.832	-23.079							
TSCR											

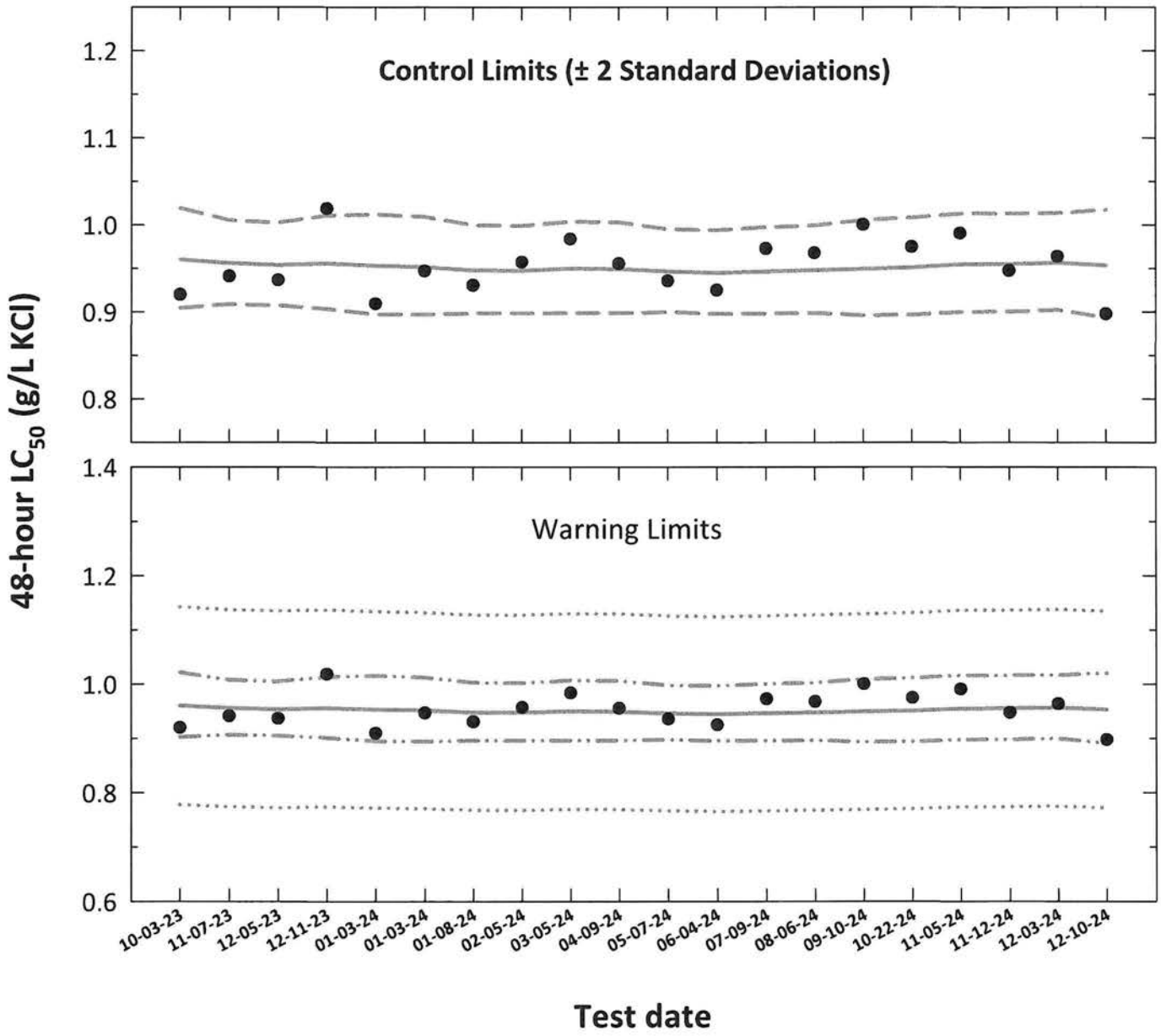
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	675.376	530.048	760.01
EC05	3.355	749.516	623.088	823.667
EC10	3.718	792.311	678.261	860.931
EC15	3.964	822.556	717.57	887.825
EC20	4.158	847.416	749.867	910.479
EC25	4.326	869.341	778.185	931.029
EC40	4.747	927.137	850.816	989.008
EC50	5.000	963.741	894.12	1029.76
EC60	5.253	1001.79	936.015	1076.33
EC75	5.674	1068.39	1001.41	1168.44
EC80	5.842	1096.03	1026.06	1210.14
EC85	6.036	1129.16	1054.15	1262.32
EC90	6.282	1172.26	1088.91	1333.24
EC95	6.645	1239.2	1140.03	1448.93
EC99	7.326	1375.23	1237.51	1700.51



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	**10-03-23**	**0.9201**	-0.0362	-0.0175	0.0129	0.9604	0.9049	1.0194	0.9026	1.0219	0.7780	1.1429
2	**11-07-23**	**0.9413**	-0.0263	-0.0195	0.0109	0.9561	0.9092	1.0055	0.9071	1.0078	0.7745	1.1378
3	**12-05-23**	**0.9368**	-0.0283	-0.0204	0.0108	0.9541	0.9077	1.0028	0.9054	1.0052	0.7728	1.1353
4	**12-11-23**	**1.0186**	0.0080	-0.0197	0.0122	0.9556	0.9035	1.0107	0.9011	1.0133	0.7740	1.1372
5	**01-03-24**	**0.9095**	-0.0412	-0.0208	0.0131	0.9532	0.8974	1.0124	0.8947	1.0153	0.7721	1.1343
6	**01-03-24**	**0.9470**	-0.0236	-0.0216	0.0128	0.9516	0.8971	1.0093	0.8943	1.0123	0.7708	1.1323
7	**01-08-24**	**0.9307**	-0.0312	-0.0233	0.0116	0.9478	0.8985	0.9999	0.8958	1.0027	0.7678	1.1279
8	**02-05-24**	**0.9572**	-0.0190	-0.0234	0.0115	0.9475	0.8986	0.9992	0.8958	1.0020	0.7675	1.1276
9	**03-05-24**	**0.9839**	-0.0070	-0.0223	0.0120	0.9499	0.8988	1.0039	0.8961	1.0067	0.7694	1.1304
10	**04-09-24**	**0.9556**	-0.0197	-0.0225	0.0119	0.9495	0.8987	1.0031	0.8961	1.0060	0.7691	1.1299
11	**05-07-24**	**0.9358**	-0.0288	-0.0239	0.0109	0.9465	0.9002	0.9952	0.8976	0.9979	0.7667	1.1263
12	**06-04-24**	**0.9251**	-0.0338	-0.0246	0.0110	0.9449	0.8980	0.9942	0.8953	0.9970	0.7653	1.1244
13	**07-09-24**	**0.9729**	-0.0119	-0.0238	0.0114	0.9467	0.8985	0.9975	0.8958	1.0004	0.7668	1.1266
14	**08-06-24**	**0.9680**	-0.0141	-0.0232	0.0115	0.9481	0.8991	0.9998	0.8964	1.0026	0.7679	1.1282
15	**09-10-24**	**1.0007**	0.0003	-0.0224	0.0125	0.9496	0.8964	1.0060	0.8936	1.0090	0.7692	1.1301
16	**10-22-24**	**0.9751**	-0.0109	-0.0217	0.0128	0.9513	0.8970	1.0088	0.8942	1.0118	0.7705	1.1320
17	**11-05-24**	**0.9904**	-0.0042	-0.0201	0.0128	0.9548	0.9000	1.0130	0.8974	1.0157	0.7734	1.1362
18	**11-12-24**	**0.9473**	-0.0235	-0.0200	0.0128	0.9551	0.9005	1.0130	0.8979	1.0157	0.7736	1.1366
19	**12-03-24**	**0.9637**	-0.0160	-0.0193	0.0127	0.9565	0.9023	1.0138	0.8998	1.0165	0.7747	1.1382
20	**12-10-24**	**0.8977**	-0.0469	-0.0207	0.0141	0.9534	0.8936	1.0172	0.8906	1.0203	0.7722	1.1345

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 # 173

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

Stock solution INSS #: 2334

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	[illegible]	[illegible]	[illegible]
Control, MHSW	pH (S.U.)	7.31	7.34	7.33
	Dissolved oxygen (mg/L)	7.8	7.2	7.2
	Conductivity (µmhos/cm)	311		
	Alkalinity (mg/L $CaCO_3$)	59		
	Hardness (mg/L $CaCO_3$)	84		
	Temperature (°C)	25.2	24.9	25.0
500 mg/L	pH (S.U.)	7.41	7.48	7.51
	Dissolved oxygen (mg/L)	7.9	7.3	7.3
	Conductivity (µmhos/cm)	1160		
	Temperature (°C)	24.4	25.1	24.7
750 mg/L	pH (S.U.)	7.49	7.47	7.52
	Dissolved oxygen (mg/L)	7.9	7.2	7.3
	Conductivity (µmhos/cm)	1610		
	Temperature (°C)	24.2	24.8	24.7
1000 mg/L	pH (S.U.)	7.53	7.47	7.56
	Dissolved oxygen (mg/L)	8.0	7.2	7.3
	Conductivity (µmhos/cm)	2050		
	Temperature (°C)	24.4	24.8	24.7
1250 mg/L	pH (S.U.)	7.55	7.47	
	Dissolved oxygen (mg/L)	8.0	7.1	
	Conductivity (µmhos/cm)	2480		
	Temperature (°C)	24.1	24.7	
1500 mg/L	pH (S.U.)	7.57	7.49	
	Dissolved oxygen (mg/L)	8.0	7.0	
	Conductivity (µmhos/cm)	2870		
	Temperature (°C)	24.2	25.0	

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

Chemical analyses:

Parameter	Reporting limit	Method number	Meter	Serial numbe
pH	0.1 S.U.	SM 4500-H+ B-2021	Accumet AR20	93312452
Dissolved oxygen	1.0 mg/L	SM 4500-O G-2021	YSI Model 52CE	08A100271
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 applicabl
Temperature	0.1 °C	SM 2550B-2010	Digital Thermometer	13066

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 # 173

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	12-11-24	* 0600	JP	0840	JP	6 B	orange	12-06-24
24	12-12-24			0837	JP			
48 Termination	12-13-24			0815	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:	11-27-24
Age (1 to 14 days old):	8 to 9 days old
Hatch date and times:	12-02-24 1452 to 12-03-24 0500
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.50
	Temperature (°C): 24.5 °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	8 2d	9 1d	2 8d	4 6d	0 10d	0 10d	0 10d	0 10d
48 Termination	10	10	10	10 1bs	8	9	2	4	0	0	0	0
Mean Survival	100%		100%		85%		30%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	829.8
Upper 95% confidence limit (mg KCl/L)	962.6
48-hour LC_{50} (mg KCl/L)	897.7

Comments:

Test Reviewed by: A

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 and 48 Hr Survival

Start Date:	12/10/2024	Test ID:	PpKClAC	Sample ID:	PpKClAC
End Date:	12/13/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2
D-Control	1.0000	1.0000
500	1.0000	1.0000
750	0.8000	0.9000
1000	0.2000	0.4000
1250	0.0000	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	0.2368	0	20
750	0.8500	0.8500	1.1781	1.1071	1.2490	8.517	2	2.816	2.850	0.2368	3	20
*1000	0.3000	0.3000	0.5742	0.4636	0.6847	27.225	2	10.086	2.850	0.2368	14	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	15.086	2.850	0.2368	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

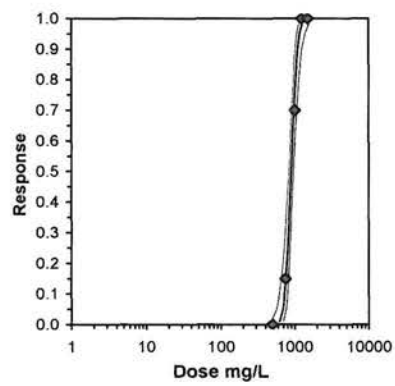
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.12346	0.12662	0.62308	0.0069	7.6E-05	4, 5
Treatments vs D-Control										

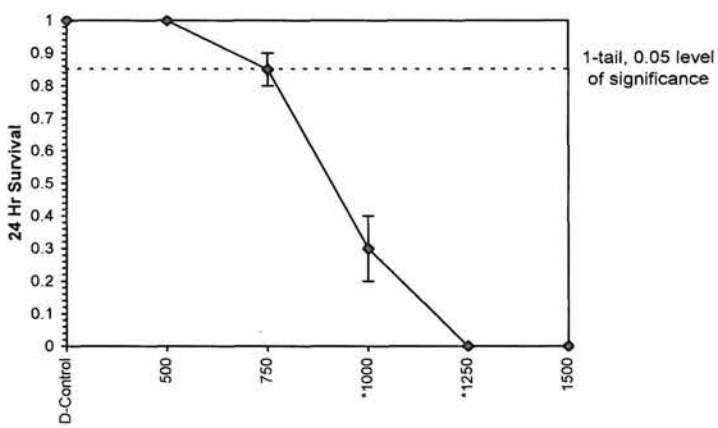
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.5067	2.82418	8.97127	20.0421	0	0.75848	7.81472	0.85937	2.95313	0.06893	4
Intercept	-37.84	8.37504	-54.255	-21.425							
TSCR											

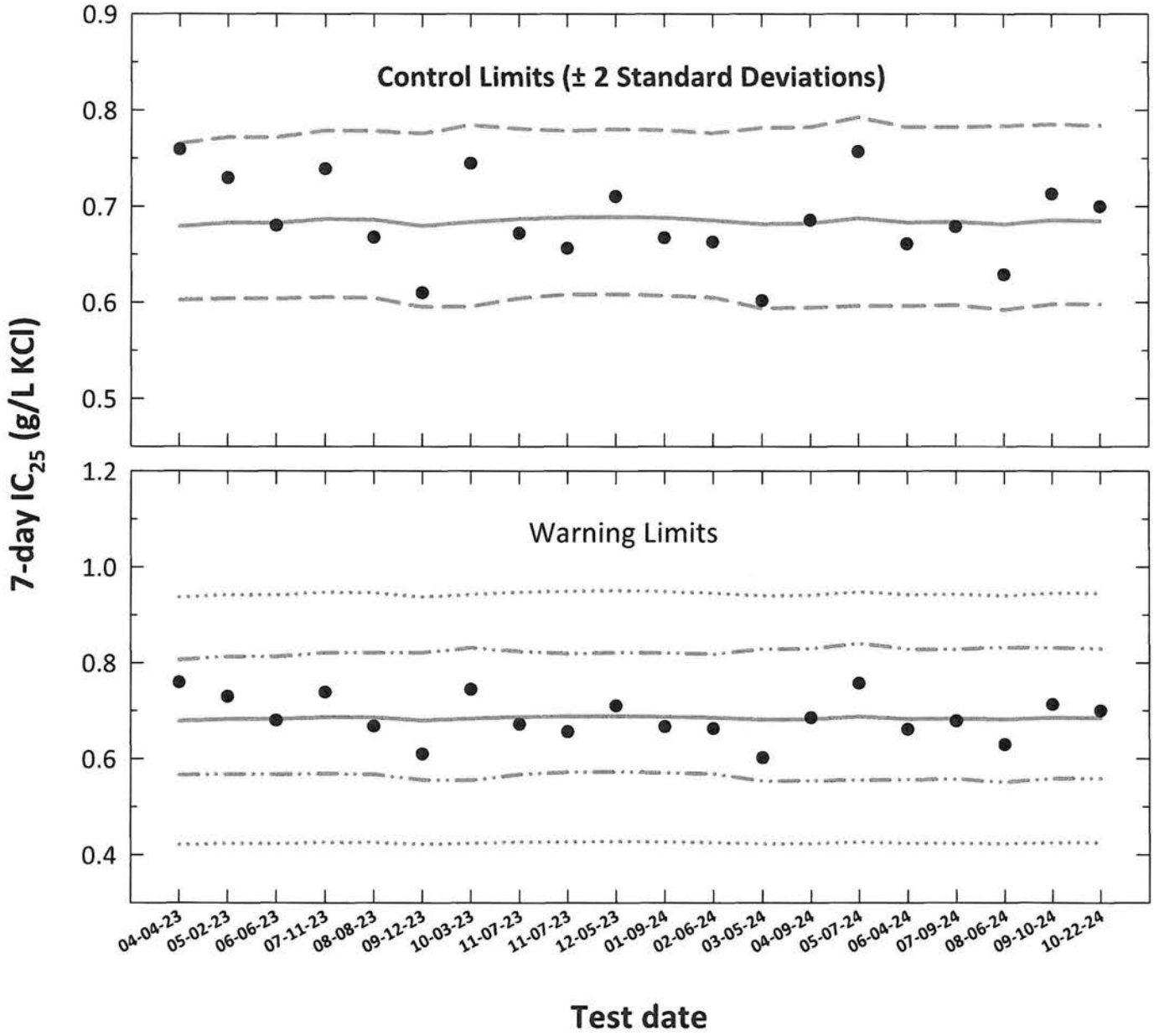
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	620.535	479.15	702.328
EC05	3.355	691.424	567.969	763.229
EC10	3.718	732.467	620.959	798.985
EC15	3.964	761.527	658.839	824.852
EC20	4.158	785.442	690.03	846.686
EC25	4.326	806.557	717.42	866.534
EC40	4.747	862.316	787.787	922.797
EC50	5.000	897.698	829.753	962.602
EC60	5.253	934.533	870.307	1008.33
EC75	5.674	999.138	933.487	1099.32
EC80	5.842	1026	957.275	1140.68
EC85	6.036	1058.22	984.389	1192.53
EC90	6.282	1100.2	1017.94	1263.18
EC95	6.645	1165.51	1067.35	1378.81
EC99	7.326	1298.66	1161.74	1631.82



Dose-Response Plot



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



- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- — — — **Control Limits** (mean logarithmic IC_{25} ± 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

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	04-04-23	**0.7592**	-0.1196	-0.1680	0.0260	0.6792	0.6025	0.7656	0.5663	0.8064	0.4211	0.9373
2	05-02-23	**0.7295**	-0.1370	-0.1656	0.0266	0.6829	0.6042	0.7718	0.5677	0.8131	0.4234	0.9424
3	06-06-23	**0.6800**	-0.1675	-0.1657	0.0266	0.6828	0.6041	0.7717	0.5676	0.8130	0.4233	0.9422
4	07-11-23	**0.7387**	-0.1315	-0.1632	0.0273	0.6868	0.6056	0.7789	0.5686	0.8208	0.4258	0.9478
5	08-08-23	**0.6677**	-0.1754	-0.1637	0.0274	0.6860	0.6045	0.7784	0.5673	0.8207	0.4253	0.9467
6	09-12-23	**0.6098**	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
7	10-03-23	**0.7447**	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436
8	11-07-23	**0.6718**	-0.1728	-0.1631	0.0278	0.6869	0.6045	0.7806	0.5669	0.8232	0.4259	0.9479
9	11-07-23	**0.6562**	-0.1830	-0.1622	0.0268	0.6883	0.6085	0.7786	0.5724	0.8194	0.4268	0.9499
10	12-05-23	**0.7102**	-0.1486	-0.1617	0.0269	0.6891	0.6088	0.7801	0.5725	0.8211	0.4273	0.9510
11	01-09-24	**0.6671**	-0.1758	-0.1624	0.0271	0.6880	0.6073	0.7795	0.5707	0.8209	0.4266	0.9495
12	02-06-24	**0.6628**	-0.1786	-0.1640	0.0270	0.6854	0.6052	0.7762	0.5684	0.8179	0.4250	0.9459
13	03-05-24	**0.6019**	-0.2205	-0.1666	0.0298	0.6814	0.5940	0.7817	0.5531	0.8286	0.4225	0.9404
14	04-09-24	**0.6855**	-0.1640	-0.1660	0.0298	0.6823	0.5949	0.7825	0.5542	0.8292	0.4230	0.9416
15	05-07-24	**0.7569**	-0.1210	-0.1627	0.0309	0.6876	0.5965	0.7927	0.5551	0.8404	0.4263	0.9489
16	06-04-24	**0.6610**	-0.1798	-0.1656	0.0295	0.6830	0.5963	0.7823	0.5561	0.8283	0.4234	0.9425
17	07-09-24	**0.6789**	-0.1682	-0.1650	0.0293	0.6839	0.5976	0.7826	0.5577	0.8283	0.4240	0.9438
18	08-06-24	**0.6287**	-0.2015	-0.1667	0.0304	0.6813	0.5922	0.7837	0.5506	0.8316	0.4224	0.9402
19	09-10-24	**0.7128**	-0.1470	-0.1640	0.0295	0.6856	0.5983	0.7855	0.5583	0.8313	0.4250	0.9461
20	10-22-24	**0.6997**	-0.1551	-0.1644	0.0294	0.6849	0.5983	0.7841	0.5585	0.8297	0.4247	0.9452

Note:

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

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

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

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

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

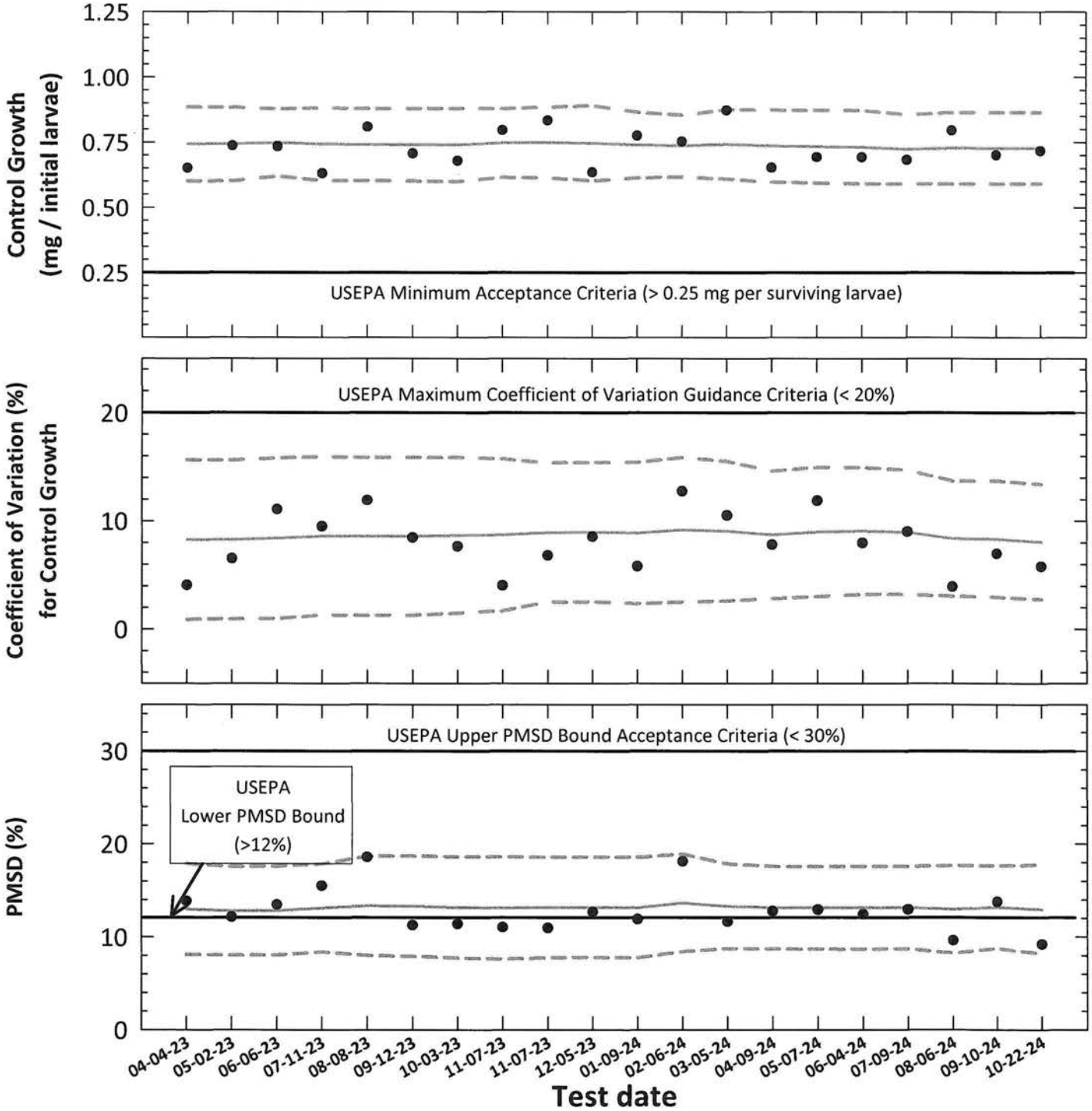
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

Central Tendency (mean Control Growth, CV or PMSD)

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival (%)	Control Growth Mean (mg/initial larvae)	Control Growth CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	04-04-23	100	0.651	4.1	0.0900	13.8	0.742	0.601	0.884	8.3	0.9	15.6	13.0	8.1	17.9
2	05-02-23	100	0.737	6.5	0.0895	12.1	0.743	0.602	0.884	8.3	1.0	15.6	12.8	8.0	17.6
3	06-06-23	100	0.734	11.1	0.0988	13.5	0.749	0.619	0.878	8.4	1.0	15.8	12.8	8.0	17.6
4	07-11-23	100	0.630	9.5	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8
5	08-08-23	100	0.809	11.9	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7
6	09-12-23	100	0.707	8.5	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7
7	10-03-23	100	0.678	7.7	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6
8	11-07-23	100	0.797	4.0	0.0880	11.0	0.748	0.616	0.880	8.7	1.7	15.8	13.1	7.6	18.6
9	11-07-23	100	0.833	6.8	0.0913	11.0	0.750	0.614	0.886	8.9	2.5	15.4	13.2	7.8	18.6
10	12-05-23	100	0.635	8.6	0.0805	12.7	0.746	0.602	0.890	9.0	2.5	15.4	13.2	7.8	18.6
11	01-09-24	100	0.776	5.8	0.0923	11.9	0.740	0.614	0.866	8.9	2.4	15.4	13.1	7.7	18.6
12	02-06-24	100	0.753	12.8	0.1364	18.1	0.736	0.618	0.855	9.2	2.5	15.9	13.7	8.4	18.9
13	03-05-24	100	0.873	10.5	0.1016	11.6	0.742	0.609	0.876	9.1	2.6	15.5	13.3	8.7	17.8
14	04-09-24	100	0.653	7.8	0.0834	12.8	0.736	0.598	0.874	8.7	2.8	14.6	13.1	8.7	17.6
15	05-07-24	100	0.693	11.9	0.0896	12.9	0.734	0.595	0.874	9.0	3.0	15.0	13.1	8.7	17.6
16	06-04-24	100	0.693	8.0	0.0862	12.4	0.732	0.591	0.872	9.1	3.2	14.9	13.1	8.7	17.6
17	07-09-24	100	0.683	9.0	0.0883	12.9	0.724	0.591	0.857	9.0	3.2	14.7	13.1	8.7	17.6
18	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	8.3	17.7
19	09-10-24	100	0.701	7.0	0.0964	13.8	0.728	0.591	0.865	8.3	2.9	13.7	13.2	8.7	17.6
20	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.9	8.2	17.7

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

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

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

MSD = Minimum significant difference.

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

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: **120**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2319				
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:	10-16-24	*Artemia* CHM number:	CHM1294
Hatch dates and times:	10-21-24 1515 TO 10-22-24 0430	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.02 S.U. Temperature = 24.2 °C	Date / Time in oven:	10-29-24 0635
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	10-30-24 0635
		*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	10-22-24	0500	JH	1230	JH	0711	JH	10-20-24
1	10-23-24	0500	JH	1230	JH	0710	JH	↓
2	10-24-24	0500	JH	1230	JH	0710	JH	10-21-24
3	10-25-24	0500	JH	1220	JH	0700	JH	↓
4	10-26-24	0700	JH	1300	JH	0900	JH	10-23-24
5	10-27-24	0600	JH	1200	JH	0800	JH	↓
6	10-28-24	0500	JH	1100	JH	0700	JH	↓
7	10-29-24					0512	JH	

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 G-2021	YSI Model 52CE	08A100271
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:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	871.3
Average weight per initial larvae:	0.717		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.717	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	699.7

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **120**

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: Forest Green Analyst: EM Date: 09-23-24	14.44	13.56	13.19	13.28	13.53	13.94	13.77	13.05	13.30	13.68	13.69	14.31
*B = Pan + Larvae weight (mg) Analyst: JH Date: 10.30.24	21.08	20.70	20.84	20.52	22.25	22.17	21.90	21.12	20.08	21.44	20.65	21.35
C = Larvae weight (mg) = B – A Analyst: JH	6.64	7.14	7.65	7.24	8.72	8.23	8.13	8.07	6.78	7.76	6.96	7.04
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.664	0.714	0.765	0.724	0.872	0.823	0.813	0.807	0.678	0.776	0.696	0.704
Average weight per initial number of larvae (mg)	0.717				0.829				0.714			
Percent reduction from control (%)							-15.6%				0.5%	

*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: **120**

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	9 1d	10	10	10
2	10	10	10	10	10	10	9 1d	9 1d	8 1d	9 1d	9 1d	9 1d
3	10	10	10	10	10	10	9	9	8	9	8 1d	8 1d
4	10	10	10	10	9 1d	9 1d	8 1d	9	8	7 2d	8	7 1d
5	10	10	10	10	7 2d	8 1d	8	9	6 2d	6 1d	7 1d	7
6	10	10	10	10	6 1d	8	8	8 1d	5 1d	5 1d	7	7
7	10	10	10	9 1d	6	8	8	8	5	5	7	6 1d
*A = Pan weight (mg) Tray color code: Forest Green Analyst: PM Date: 09-23-24	11.94	14.06	11.72	14.19	14.34	13.62	14.26	14.39	15.63	13.58	13.77	11.83
*B = Pan + Larvae weight (mg) Analyst: JH Date: 10-30-24	17.99	20.86	18.88	20.99	18.61	19.93	19.22	20.20	19.11	17.34	18.75	16.90
C = Larvae weight (mg) = B – A Analyst: JH	6.05	6.80	7.16	6.80	4.27	6.31	4.96	5.81	3.48	3.76	4.98	5.07
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.605	0.680	0.716	0.680	0.427	0.631	0.496	0.581	0.348	0.376	0.498	0.507
Average weight per initial number of larvae (mg)	0.670				0.534				0.432			
Percent reduction from control (%)			6.5%				25.5%				39.7%	

*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: **120**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	8 2d	8 2d	8 2d	7 3d
2	6 2d	4 4d	4 4d	6 1d
3	5 1d	4	4	4 2d
4	3 2d	2 2d	2 2d	4
5	1 2d	1 1d	2	2 2d
6	0 1d	0 1d	2	2
7	0	0	2	1 1d
*A = Pan weight (mg) Tray color code: Forest Green Analyst: EM Date: ~~09-13-24~~ ~~09-23-24~~ 09-23-24	14.72	12.44	14.01	14.65
*B = Pan + Larvae weight (mg) Analyst: JM Date: 10-30-24	—	JM 10-30-24	15.62	15.50
C = Larvae weight (mg) = B – A Analyst: JM	—	JM 10-30-24	1.61	0.85
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JM	0	0	0.161	0.085
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.062		91.4 %	

*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: 120

Test dates: October 22-29, 2024

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	14.44	21.08	6.64	0.664	0.717	5.8	0.664	100.0	0.717	5.8	Not applicable
	B	10	10	13.56	20.70	7.14	0.714			0.714				
	C	10	10	13.19	20.84	7.65	0.765			0.765				
	D	10	10	13.28	20.52	7.24	0.724			0.724				
300	E	10	10	13.53	22.25	8.72	0.872	0.829	3.6	0.872	100.0	0.829	3.6	-15.6
	F	10	10	13.94	22.17	8.23	0.823			0.823				
	G	10	10	13.77	21.90	8.13	0.813			0.813				
	H	10	10	13.05	21.12	8.07	0.807			0.807				
450	I	10	10	13.30	20.08	6.78	0.678	0.714	6.0	0.678	100.0	0.714	6.0	0.5
	J	10	10	13.68	21.44	7.76	0.776			0.776				
	K	10	10	13.69	20.65	6.96	0.696			0.696				
	L	10	10	14.31	21.35	7.04	0.704			0.704				
600	M	10	10	11.94	17.99	6.05	0.605	0.689	9.3	0.605	97.5	0.670	7.0	6.5
	N	10	10	14.06	20.86	6.80	0.680			0.680				
	O	10	10	11.72	18.88	7.16	0.716			0.716				
	P	10	9	14.19	20.99	6.80	0.756			0.680				
750	Q	10	6	14.34	18.61	4.27	0.712	0.712	9.8	0.427	75.0	0.534	16.9	25.5
	R	10	8	13.62	19.93	6.31	0.789			0.631				
	S	10	8	14.26	19.22	4.96	0.620			0.496				
	T	10	8	14.39	20.20	5.81	0.726			0.581				
900	U	10	5	15.63	19.11	3.48	0.696	0.751	8.9	0.348	57.5	0.432	19.0	39.7
	V	10	5	13.58	17.34	3.76	0.752			0.376				
	W	10	7	13.77	18.75	4.98	0.711			0.498				
	X	10	6	11.83	16.90	5.07	0.845			0.507				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.828	3.8	0.000	7.5	0.062	126.0	91.4
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	2	14.01	15.62	1.61	0.805			0.161				
	BB	10	1	14.65	15.50	0.85	0.850			0.085				

Dunnett's MSD value: 0.0659 MSD = Minimum Significant Difference

PMSD: 9.2 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	10/22/2024	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	10/29/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	0.9000
750	0.6000	0.8000	0.8000	0.8000
900	0.5000	0.5000	0.7000	0.6000
1050	0.0000	0.0000	0.2000	0.1000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9750	0.9750	1.3713	1.2490	1.4120	5.942	4	16.00	10.00	1	40
*750	0.7500	0.7500	1.0519	0.8861	1.1071	10.508	4	10.00	10.00	10	40
*900	0.5750	0.5750	0.8620	0.7854	0.9912	11.405	4	10.00	10.00	17	40
*1050	0.0750	0.0750	0.2757	0.1588	0.4636	53.294	4	10.00	10.00	37	40

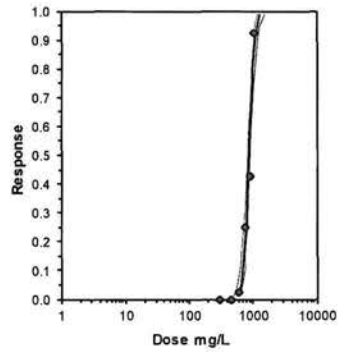
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90472	0.896	-0.0838	1.09361
Equality of variance cannot be confirmed				

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

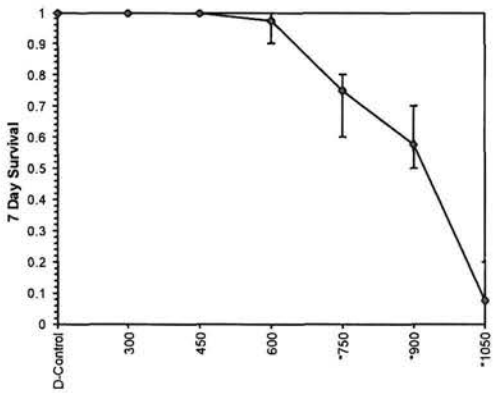
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.8124	1.75256	9.37739	16.2474	0	5.9112	9.48773	0.20588	2.94017	0.07805	3
Intercept	-32.671	5.14602	-42.757	-22.585							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	573.593	490.051	630.34
EC05	3.355	648.327	577.483	696.459
EC10	3.718	692.069	629.586	735.332
EC15	3.964	723.237	666.817	763.409
EC20	4.158	749.007	697.456	787.068
EC25	4.326	771.845	724.329	808.542
EC40	4.747	832.531	793.034	869.315
EC50	5.000	871.312	833.736	912.117
EC60	5.253	911.9	873.243	960.626
EC75	5.674	983.597	936.91	1053.96
EC80	5.842	1013.59	961.974	1095.13
EC85	6.036	1049.7	991.347	1145.96
EC90	6.282	1096.98	1028.8	1214.19
EC95	6.645	1170.99	1085.84	1324.21
EC99	7.326	1323.56	1199.35	1560.98



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumser

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	10/22/2024	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	10/29/2024	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	0.6640	0.7140	0.7650	0.7240
300	0.8720	0.8230	0.8130	0.8070
450	0.6780	0.7760	0.6960	0.7040
600	0.6050	0.6800	0.7160	0.6800
750	0.4270	0.6310	0.4960	0.5810
900	0.3480	0.3760	0.4980	0.5070
1050	0.0000	0.0000	0.1610	0.0850

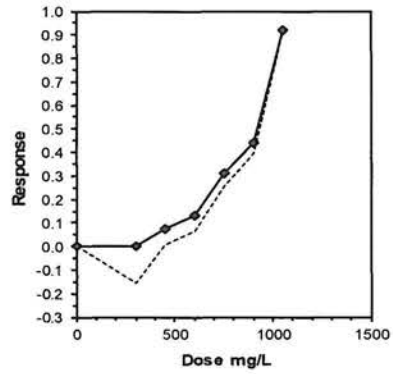
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7168	1.0000	0.7168	0.6640	0.7650	5.792	4				0.7728	1.0000
300	0.8288	1.1563	0.8288	0.8070	0.8720	3.569	4	-3.890	2.290	0.0659	0.7728	1.0000
450	0.7135	0.9955	0.7135	0.6780	0.7760	6.035	4	0.113	2.290	0.0659	0.7135	0.9233
600	0.6703	0.9351	0.6703	0.6050	0.7160	6.967	4	1.615	2.290	0.0659	0.6703	0.8674
750	0.5338	0.7447	0.5338	0.4270	0.6310	16.935	4				0.5338	0.6907
900	0.4323	0.6031	0.4323	0.3480	0.5070	18.971	4				0.4323	0.5594
1050	0.0615	0.0858	0.0615	0.0000	0.1610	126.010	4				0.0615	0.0796

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96347	0.844	0.07161	-0.5144
Bartlett's Test indicates equal variances (p = 0.90)	0.56728	11.3449		

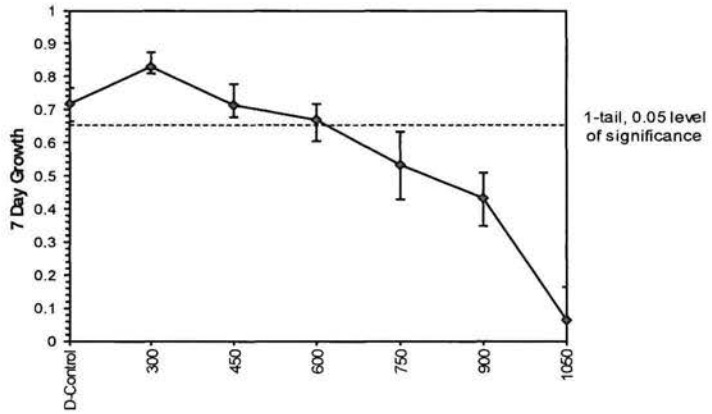
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.06594	0.092	0.01833	0.00166	9.1E-04	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	397.82	34.93	339.09	553.39	1.1722
IC10	512.51	55.60	368.06	666.04	0.1451
IC15	614.74	31.39	492.22	686.59	-0.7500
IC20	657.20	29.53	588.10	785.30	0.9253
IC25	699.66	32.02	630.47	819.13	0.6023
IC40	853.60	42.85	687.31	948.18	-0.5160
IC50	918.56	17.14	837.82	950.45	-1.3223



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **120**

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.

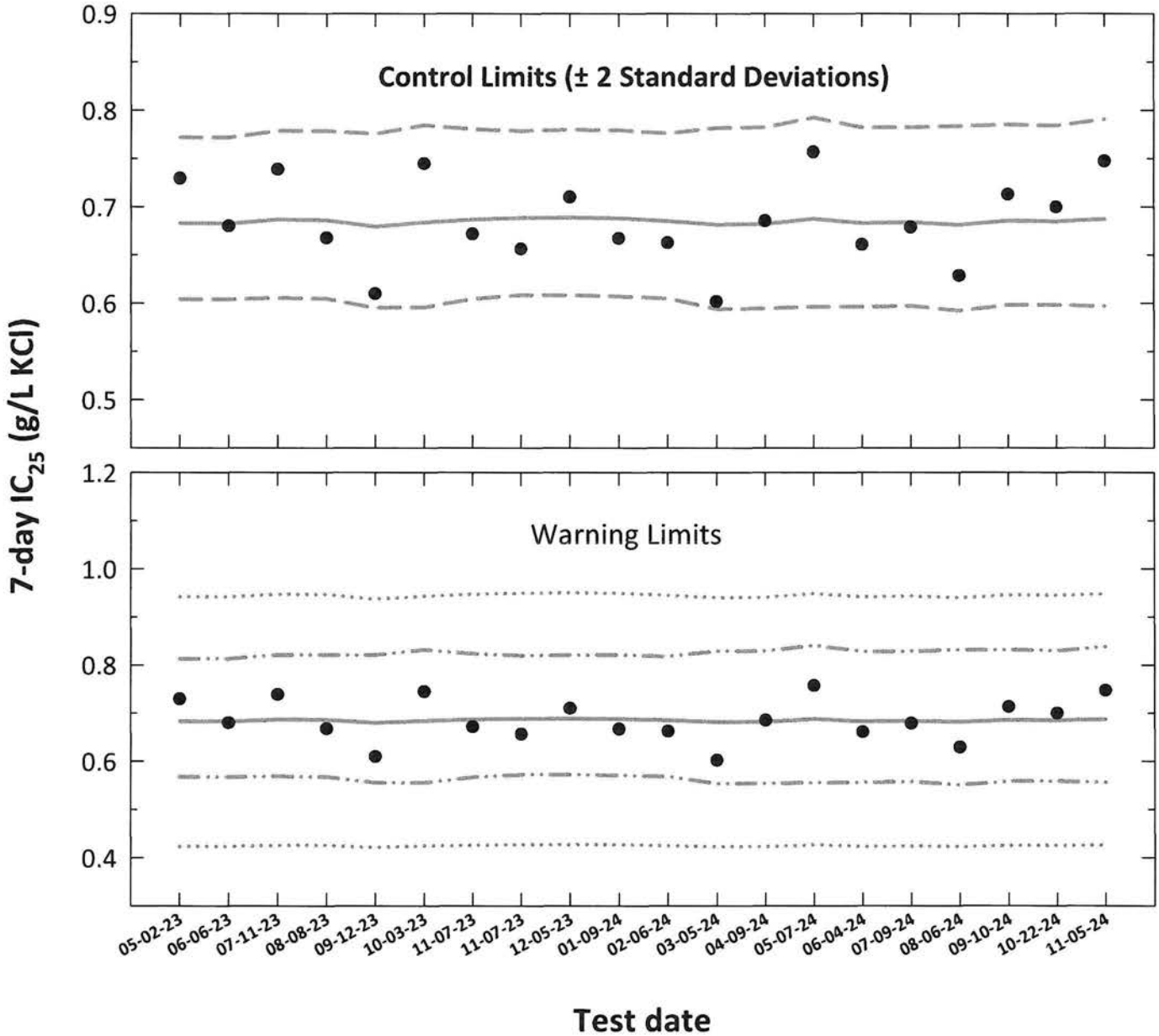
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	N	BM	BM	BM	BM	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.94	7.23	7.43	7.34	7.61	7.50
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	7.6	7.7	7.5
	Conductivity (µmhos/cm)	307		313		312	
	Alkalinity (mg $CaCO_3$/L)	58				58	
	Hardness (mg $CaCO_3$/L)	84				86	
	Temperature (°C)	24.9	24.7	24.7	24.4	24.7	24.8
300 mg KCl/L	pH (S.U.)	7.98	7.32	7.51	7.45	7.68	7.40
	Dissolved oxygen (mg/L)	7.7	7.6	7.8	7.5	7.7	7.6
	Conductivity (µmhos/cm)	811		853		855	
	Temperature (°C)	24.8	24.6	24.8	24.7	24.8	24.6
450 mg KCl/L	pH (S.U.)	7.96	7.37	7.55	7.47	7.71	7.50
	Dissolved oxygen (mg/L)	7.8	7.5	7.8	7.4	7.7	7.6
	Conductivity (µmhos/cm)	1050		1110		1110	
	Temperature (°C)	24.8	24.8	24.8	24.7	24.8	24.6
600 mg KCl/L	pH (S.U.)	7.95	7.38	7.57	7.47	7.73	7.55
	Dissolved oxygen (mg/L)	7.8	7.4	7.7	7.4	7.7	7.6
	Conductivity (µmhos/cm)	1300		1360		1380	
	Temperature (°C)	24.8	24.8	24.7	24.6	24.8	24.6
750 mg KCl/L	pH (S.U.)	7.93	7.41	7.60	7.50	7.74	7.56
	Dissolved oxygen (mg/L)	7.8	7.4	7.7	7.4	7.8	7.6
	Conductivity (µmhos/cm)	1530		1630		1630	
	Temperature (°C)	24.7	24.7	24.7	24.6	24.7	24.9
900 mg KCl/L	pH (S.U.)	7.94	7.41	7.62	7.55	7.75	7.61
	Dissolved oxygen (mg/L)	7.9	7.4	7.7	7.5	7.7	7.6
	Conductivity (µmhos/cm)	1770		1900		1890	
	Temperature (°C)	24.7	24.7	24.9	24.6	24.7	24.5
1050 mg KCl/L	pH (S.U.)	7.95	7.51	7.62	7.55	7.75	7.60
	Dissolved oxygen (mg/L)	7.9	7.4	7.6	7.4	7.8	7.7
	Conductivity (µmhos/cm)	2030		2130		2120	
	Temperature (°C)	24.9	24.6	24.7	24.5	24.8	24.9
		Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **120**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	K	K	K	K	K	K	K	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.73	7.24	7.75	7.11	7.81	7.41	7.89	7.30
	Dissolved oxygen (mg/L)	7.7	6.8	7.7	6.8	8.0	7.5	8.0	7.6
	Conductivity (µmhos/cm)	293		296		307		300	
	Alkalinity (mg $CaCO_3$/L)			57					
	Hardness (mg $CaCO_3$/L)	K 10-26-24		86				K 10-26-24	
	Temperature (°C)	24.8	24.5	24.8	24.7	25.1	25.2	24.7	24.4
300 mg KCl/L	pH (S.U.)	7.81	7.28	7.83	7.13	7.86	7.37	7.89	7.29
	Dissolved oxygen (mg/L)	7.7	6.8	7.7	6.4	8.0	7.6	7.9	7.6
	Conductivity (µmhos/cm)	807		864		846		822	
	Temperature (°C)	24.7	24.9	24.9	24.9	25.1	25.0	24.8	24.7
450 mg KCl/L	pH (S.U.)	7.81	7.35	7.85	7.13	7.86	7.38	7.89	7.33
	Dissolved oxygen (mg/L)	7.7	6.8	7.7	6.3	8.0	7.6	7.9	7.4
	Conductivity (µmhos/cm)	1050		1120		1100		1070	
	Temperature (°C)	24.8	24.9	25.0	24.9	25.0	24.9	24.8	24.6
600 mg KCl/L	pH (S.U.)	7.84	7.24	7.86	7.12	7.86	7.36	7.90	7.36
	Dissolved oxygen (mg/L)	7.8	6.8	7.7	6.2	8.0	7.5	7.9	7.4
	Conductivity (µmhos/cm)	1290		1350		1350		1320	
	Temperature (°C)	24.8	24.7	25.0	24.6	25.0	24.9	24.8	24.6
750 mg KCl/L	pH (S.U.)	7.85	7.28	7.87	7.21	7.89	7.35	7.92	7.37
	Dissolved oxygen (mg/L)	7.8	6.5	7.7	6.3	8.1	7.5	7.9	7.4
	Conductivity (µmhos/cm)	1550		1450		1620		1690	
	Temperature (°C)	24.9	24.7	25.0	24.9	25.0	24.9	24.8	24.6
900 mg KCl/L	pH (S.U.)	7.86	7.34	7.87	7.20	7.92	7.34	7.93	7.43
	Dissolved oxygen (mg/L)	7.9	6.6	7.8	6.4	8.0	7.4	8.0	7.6
	Conductivity (µmhos/cm)	1820		1850		1870		1850	
	Temperature (°C)	24.9	24.7	25.0	24.9	25.0	25.1	24.7	24.9
1050 mg KCl/L	pH (S.U.)	7.87	7.32	7.88	7.18	7.92	7.41	7.95	7.41
	Dissolved oxygen (mg/L)	7.9	6.7	7.8	6.4	8.0	7.4	8.0	7.7
	Conductivity (µmhos/cm)	2070		2050		2090		2040	
	Temperature (°C)	24.9	24.8	24.9	25.0	24.9	24.8	24.7	24.7
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the potassium chloride concentration which would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- — — — **Control Limits** (mean logarithmic IC_{25} ± 2 standard deviations converted to anti-logarithmic values)
- —·—·— **Laboratory Warning Limits** (mean logarithmic IC_{25} ± 2 coefficent of variations converted to anti-logarithmic values)
- ····· **USEPA Warning Limits** (mean logarithmic IC_{25} ± $S_{A.75}$ 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	05-02-23	**0.7295**	-0.1370	-0.1656	0.0266	0.6829	0.6042	0.7718	0.5677	0.8131	0.4234	0.9424
2	06-06-23	**0.6800**	-0.1675	-0.1657	0.0266	0.6828	0.6041	0.7717	0.5676	0.8130	0.4233	0.9422
3	07-11-23	**0.7387**	-0.1315	-0.1632	0.0273	0.6868	0.6056	0.7789	0.5686	0.8208	0.4258	0.9478
4	08-08-23	**0.6677**	-0.1754	-0.1637	0.0274	0.6860	0.6045	0.7784	0.5673	0.8207	0.4253	0.9467
5	09-12-23	**0.6098**	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
6	10-03-23	**0.7447**	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436
7	11-07-23	**0.6718**	-0.1728	-0.1631	0.0278	0.6869	0.6045	0.7806	0.5669	0.8232	0.4259	0.9479
8	11-07-23	**0.6562**	-0.1830	-0.1622	0.0268	0.6883	0.6085	0.7786	0.5724	0.8194	0.4268	0.9499
9	12-05-23	**0.7102**	-0.1486	-0.1617	0.0269	0.6891	0.6088	0.7801	0.5725	0.8211	0.4273	0.9510
10	01-09-24	**0.6671**	-0.1758	-0.1624	0.0271	0.6880	0.6073	0.7795	0.5707	0.8209	0.4266	0.9495
11	02-06-24	**0.6628**	-0.1786	-0.1640	0.0270	0.6854	0.6052	0.7762	0.5684	0.8179	0.4250	0.9459
12	03-05-24	**0.6019**	-0.2205	-0.1666	0.0298	0.6814	0.5940	0.7817	0.5531	0.8286	0.4225	0.9404
13	04-09-24	**0.6855**	-0.1640	-0.1660	0.0298	0.6823	0.5949	0.7825	0.5542	0.8292	0.4230	0.9416
14	05-07-24	**0.7569**	-0.1210	-0.1627	0.0309	0.6876	0.5965	0.7927	0.5551	0.8404	0.4263	0.9489
15	06-04-24	**0.6610**	-0.1798	-0.1656	0.0295	0.6830	0.5963	0.7823	0.5561	0.8283	0.4234	0.9425
16	07-09-24	**0.6789**	-0.1682	-0.1650	0.0293	0.6839	0.5976	0.7826	0.5577	0.8283	0.4240	0.9438
17	08-06-24	**0.6287**	-0.2015	-0.1667	0.0304	0.6813	0.5922	0.7837	0.5506	0.8316	0.4224	0.9402
18	09-10-24	**0.7128**	-0.1470	-0.1640	0.0295	0.6856	0.5983	0.7855	0.5583	0.8313	0.4250	0.9461
19	10-22-24	**0.6997**	-0.1551	-0.1644	0.0294	0.6849	0.5983	0.7841	0.5585	0.8297	0.4247	0.9452
20	11-05-24	**0.7473**	-0.1265	-0.1629	0.0305	0.6872	0.5971	0.7908	0.5561	0.8380	0.4261	0.9483

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

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

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

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

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

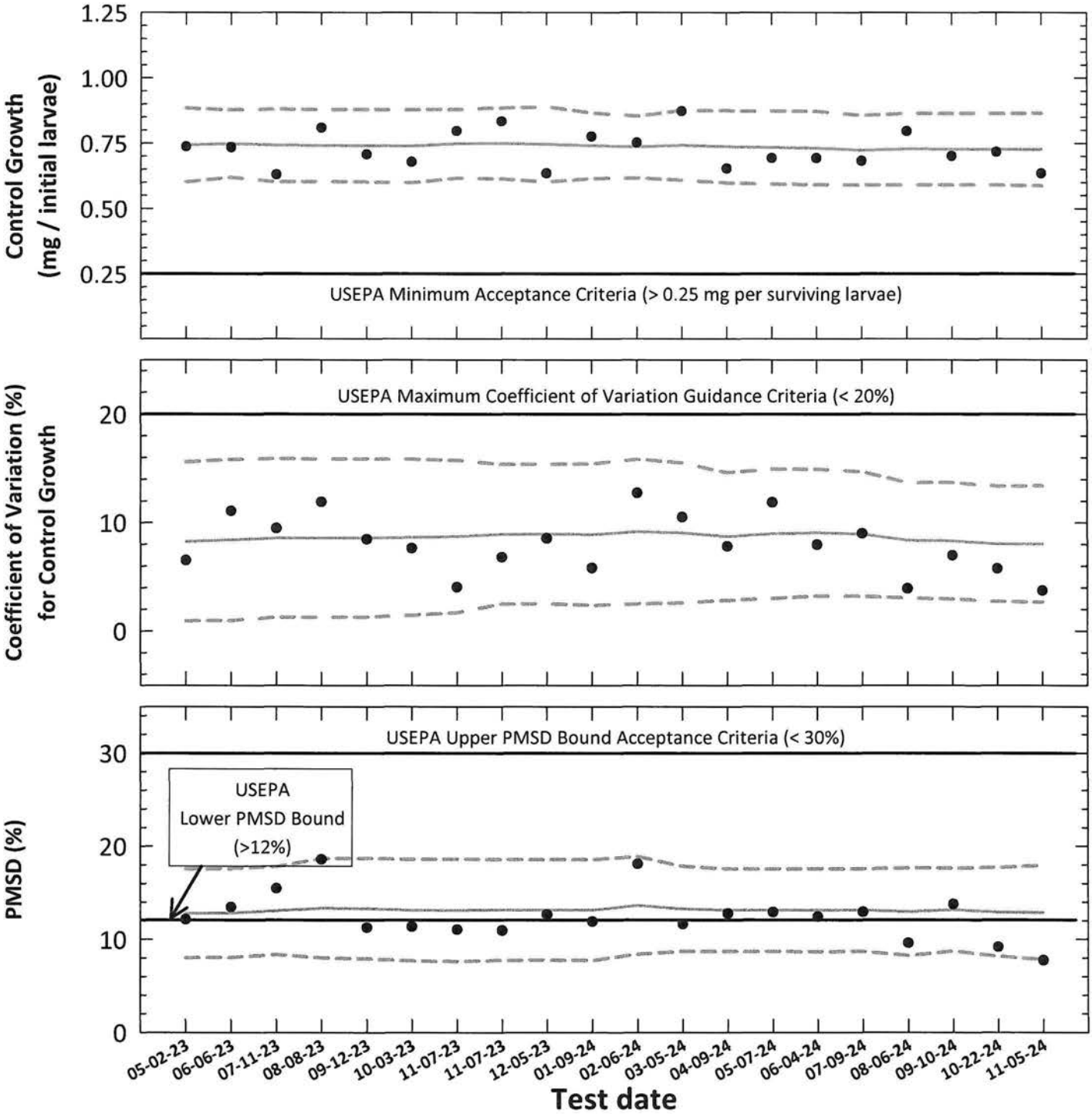
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

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

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

Entered and Reviewed by Jim Sumner

ETS Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival (%)	Control Growth Mean (mg/initial larvae)	Control Growth CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S
1	05-02-23	100	0.737	6.5	0.0895	12.1	0.743	0.602	0.884	8.3	1.0	15.6	12.8	8.0	17.6
2	06-06-23	100	0.734	11.1	0.0988	13.5	0.749	0.619	0.878	8.4	1.0	15.8	12.8	8.0	17.6
3	07-11-23	100	0.630	9.5	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8
4	08-08-23	100	0.809	11.9	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7
5	09-12-23	100	0.707	8.5	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7
6	10-03-23	100	0.678	7.7	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6
7	11-07-23	100	0.797	4.0	0.0880	11.0	0.748	0.616	0.880	8.7	1.7	15.8	13.1	7.6	18.6
8	11-07-23	100	0.833	6.8	0.0913	11.0	0.750	0.614	0.886	8.9	2.5	15.4	13.2	7.8	18.6
9	12-05-23	100	0.635	8.6	0.0805	12.7	0.746	0.602	0.890	9.0	2.5	15.4	13.2	7.8	18.6
10	01-09-24	100	0.776	5.8	0.0923	11.9	0.740	0.614	0.866	8.9	2.4	15.4	13.1	7.7	18.6
11	02-06-24	100	0.753	12.8	0.1364	18.1	0.736	0.618	0.855	9.2	2.5	15.9	13.7	8.4	18.9
12	03-05-24	100	0.873	10.5	0.1016	11.6	0.742	0.609	0.876	9.1	2.6	15.5	13.3	8.7	17.8
13	04-09-24	100	0.653	7.8	0.0834	12.8	0.736	0.598	0.874	8.7	2.8	14.6	13.1	8.7	17.6
14	05-07-24	100	0.693	11.9	0.0896	12.9	0.734	0.595	0.874	9.0	3.0	15.0	13.1	8.7	17.6
15	06-04-24	100	0.693	8.0	0.0862	12.4	0.732	0.591	0.872	9.1	3.2	14.9	13.1	8.7	17.6
16	07-09-24	100	0.683	9.0	0.0883	12.9	0.724	0.591	0.857	9.0	3.2	14.7	13.1	8.7	17.6
17	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	8.3	17.7
18	09-10-24	100	0.701	7.0	0.0964	13.8	0.728	0.591	0.865	8.3	2.9	13.7	13.2	8.7	17.6
19	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.9	8.2	17.7
20	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.9	7.8	17.9

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: 121

Dilution preparation information:							*Comments:*
KCl Stock INSS number:			INSS 2319				
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:	7A
Spawn date:	10-30-24	*Artemia* CHM number:	CHM1294
Hatch dates and times:	11-04-24 ~~11-05-24~~ 1500 TO 11-05-24 0405	*Drying information for weight determination:*	
Transfer vessel information:	pH = 7.96 S.U. Temperature = 24.4 °C	Date / Time in oven:	11-12-24 0620
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	11-13-24 0620
		*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	11-05-24	0500	JK	1130 ~~1215~~ 11-06-24	JK	0711	JK	10-30-24 A
1	11-06-24	0500	JK	1130	JK	0700	JK	↓
2	11-07-24	0500	JK	1230	JK	0700	JK	10-30-24 B
3	11-08-24	0500	JK	1100	JK	0700	JK	↓
4	11-09-24	0600	JK	1300	JK	0822	JK	11-04-24 B
5	11-10-24	0500	JK	1100	JK	0700	JK	↓
6	11-11-24	0500	JK	1100	JK	0700	JK	↓
7	11-12-24					0512	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 G-2021	YSI Model 52CE	08A100271
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:	0%	≤ 20%	7-day LC_{50} (mg/L KCl)	841.8
Average weight per initial larvae:	0.635		NOEC (mg/L KCl)	600
Average weight per surviving larvae:	0.635	≥ 0.25 mg/larvae	LOEC (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	747.3

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **121**

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: Forest Green Analyst: [illegible] Date: 10-30-24	14.04	15.89	13.40	12.61	13.60	13.92	15.10	14.45	14.26	14.23	14.95	15.34
*B = Pan + Larvae weight (mg) Analyst: EM Date: 11-14-24	20.19	22.49	19.88	18.75	21.45	20.83	21.97	21.73	20.42	20.48	21.29	21.50
C = Larvae weight (mg) = B – A Analyst: [illegible]	6.15	6.61	6.48	6.14	7.85	6.91	6.87	7.28	6.16	6.25	6.34	6.16
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: [illegible]	0.615	0.661	0.648	0.614	0.785	0.691	0.687	0.728	0.616	0.625	0.634	0.616
Average weight per initial number of larvae (mg)	0.635				0.723				0.623			
Percent reduction from control (%)							-13.9%				1.9%	

*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: **121**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	9 1d	9 1d	9 1d	7 2d	7 2d	8 2d	8 2d
3	10	10	10	10	8 2d	9	9	9	4 3d	4 3d	4 4d	4 4d
4	10	10	10	10	8	9	8 1d	9	4	4	4	4
5	10	10	10	10	8	9	8	9	4	4	4	3 1d
6	10	10	10	10	8	9	8	9	4	3 1d	4	3
7	10	10	10	10	8	8 1d	8	7 2d	2 2d	3	3 1d	3
*A = Pan weight (mg) Tray color code: Forest Green Analyst: BM Date: 12-30-24	13.17	13.91	11.81	15.44	14.77	14.66	14.67	13.49	13.79	13.45	13.77	15.57
*B = Pan + Larvae weight (mg) Analyst: BM Date: 11-14-24	19.27	20.31	17.46	21.38	19.83	20.01	20.07	17.97	14.76	15.52	15.89	17.55
C = Larvae weight (mg) = B – A Analyst: JL	6.10	6.40	5.65	5.94	5.06	5.35	5.40	4.48	0.97	2.07	2.12	1.98
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.610	0.640	0.565	0.594	0.506	0.535	0.540	0.448	0.097	0.207	0.212	0.198
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.602		5.17.		0.507		20.17.		0.179		71.97.	

*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: **121**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	7 3d	8 2d	8 2d	8 2d
2	6 1d	7 1d	7 1d	7 1d
3	3 3d	3 4d	3 4d	5 2d
4	3	3	3	5
5	3	3	3	4 1d
6	2 1d	2 1d	2 1d	3 1d
7	1 1d	1 1d	1 1d	1 2d
*A = Pan weight (mg) Tray color code: Forest + Green Analyst: AP Date: 10-30-24	12.92	12.32	14.07	15.43
*B = Pan + Larvae weight (mg) Analyst: EM Date: 11-14-24	13.69	12.80	14.52	15.75
C = Larvae weight (mg) = B – A Analyst: JL	0.77	0.48	0.45	0.32
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.077	0.048	0.045	0.032
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.051		92.07.	

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

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

Comments:

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: 121

Test dates: November 05-12, 2024

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	14.04	20.19	6.15	0.615	0.635	3.7	0.615	100.0	0.635	3.7	Not applicable
	B	10	10	15.88	22.49	6.61	0.661			0.661				
	C	10	10	13.40	19.88	6.48	0.648			0.648				
	D	10	10	12.61	18.75	6.14	0.614			0.614				
300	E	10	10	13.60	21.45	7.85	0.785	0.723	6.3	0.785	100.0	0.723	6.3	-13.9
	F	10	10	13.92	20.83	6.91	0.691			0.691				
	G	10	10	15.10	21.97	6.87	0.687			0.687				
	H	10	10	14.45	21.73	7.28	0.728			0.728				
450	I	10	10	14.26	20.42	6.16	0.616	0.623	1.4	0.616	100.0	0.623	1.4	1.9
	J	10	10	14.23	20.48	6.25	0.625			0.625				
	K	10	10	14.95	21.29	6.34	0.634			0.634				
	L	10	10	15.34	21.50	6.16	0.616			0.616				
600	M	10	10	13.17	19.27	6.10	0.610	0.602	5.2	0.610	100.0	0.602	5.2	5.1
	N	10	10	13.91	20.31	6.40	0.640			0.640				
	O	10	10	11.81	17.46	5.65	0.565			0.565				
	P	10	10	15.44	21.38	5.94	0.594			0.594				
750	Q	10	8	14.77	19.83	5.06	0.633	0.654	3.2	0.506	77.5	0.507	8.3	20.1
	R	10	8	14.66	20.01	5.35	0.669			0.535				
	S	10	8	14.67	20.07	5.40	0.675			0.540				
	T	10	7	13.49	17.97	4.48	0.640			0.448				
900	U	10	2	13.79	14.76	0.97	0.485	0.635	16.1	0.097	27.5	0.179	30.6	71.9
	V	10	3	13.45	15.52	2.07	0.690			0.207				
	W	10	3	13.77	15.89	2.12	0.707			0.212				
	X	10	3	15.57	17.55	1.98	0.660			0.198				
1050	Y	10	1	12.92	13.69	0.77	0.770	0.505	37.6	0.077	10.0	0.051	37.6	92.0
	Z	10	1	12.32	12.80	0.48	0.480			0.048				
	AA	10	1	14.07	14.52	0.45	0.450			0.045				
	BB	10	1	15.43	15.75	0.32	0.320			0.032				

Dunnett's MSD value: 0.0491

PMSD: 7.7

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	11/5/2024	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	11/12/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type.	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	1.0000	1.0000	1.0000	1.0000
750	0.8000	0.8000	0.8000	0.7000
900	0.2000	0.3000	0.3000	0.3000
1050	0.1000	0.1000	0.1000	0.1000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*750	0.7750	0.7750	1.0782	0.9912	1.1071	5.379	4	10.00	10.00	9	40
*900	0.2750	0.2750	0.5506	0.4636	0.5796	10.532	4	10.00	10.00	29	40
*1050	0.1000	0.1000	0.3218	0.3218	0.3218	0.000	4	10.00	10.00	36	40

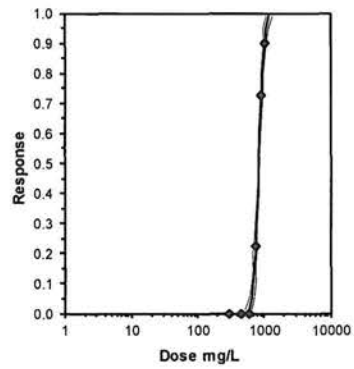
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.58279	0.896	-2.2845	6.47308
Equality of variance cannot be confirmed				

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

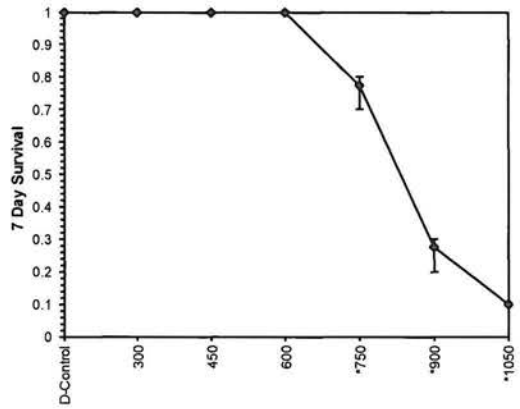
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.5065	2.04381	11.5007	19.5124	0	1.57606	9.48773	0.81309	2.92521	0.06449	4
Intercept	-40.36	5.98871	-52.098	-28.622							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	595.921	522.552	645.927
EC05	3.355	659.383	597.329	701.912
EC10	3.718	695.932	640.914	734.353
EC15	3.964	721.729	671.7	757.539
EC20	4.158	742.912	696.862	776.885
EC25	4.326	761.58	718.843	794.268
EC40	4.747	810.727	775.02	842.346
EC50	5.000	841.807	808.539	875.216
EC60	5.253	874.079	841.25	911.81
EC75	5.674	930.486	893.719	981.365
EC80	5.842	953.867	914.134	1011.86
EC85	6.036	981.863	937.859	1049.34
EC90	6.282	1018.26	967.822	1099.34
EC95	6.645	1074.7	1012.9	1179.15
EC99	7.326	1189.15	1101.06	1347.44



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	11/5/2024	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	11/12/2024	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	0.6150	0.6610	0.6480	0.6140
300	0.7850	0.6910	0.6870	0.7280
450	0.6160	0.6250	0.6340	0.6160
600	0.6100	0.6400	0.5650	0.5940
750	0.5060	0.5350	0.5400	0.4480
900	0.0970	0.2070	0.2120	0.1980
1050	0.0770	0.0480	0.0450	0.0320

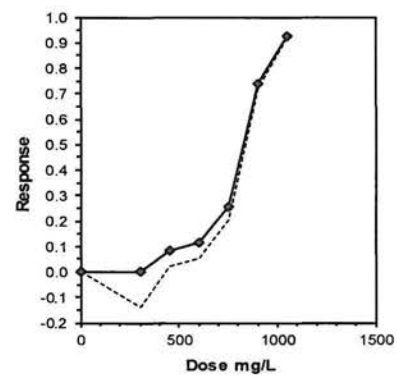
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.6345	1.0000	0.6345	0.6140	0.6610	3.735	4				0.6786	1.0000
300	0.7228	1.1391	0.7228	0.6870	0.7850	6.284	4	-4.115	2.290	0.0491	0.6786	1.0000
450	0.6228	0.9815	0.6228	0.6160	0.6340	1.384	4	0.548	2.290	0.0491	0.6228	0.9177
600	0.6023	0.9492	0.6023	0.5650	0.6400	5.199	4	1.504	2.290	0.0491	0.6023	0.8875
750	0.5073	0.7994	0.5073	0.4480	0.5400	8.329	4				0.5073	0.7475
900	0.1785	0.2813	0.1785	0.0970	0.2120	30.611	4				0.1785	0.2630
1050	0.0505	0.0796	0.0505	0.0320	0.0770	37.589	4				0.0505	0.0744

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95583	0.844	0.65482	0.43143
Bartlett's Test indicates equal variances (p = 0.13)	5.63304	11.3449		

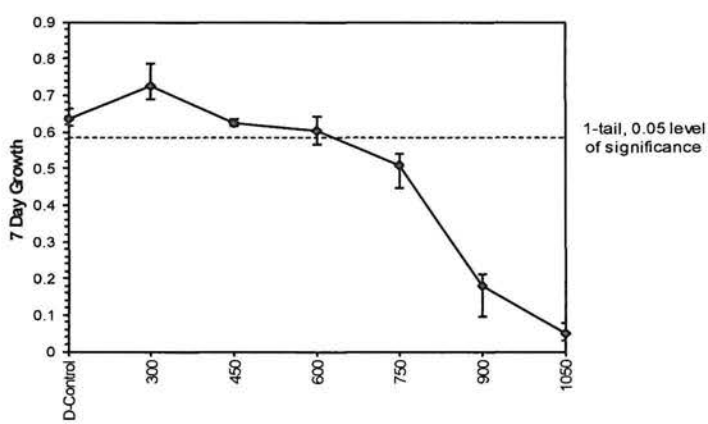
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.04911	0.0774	0.0113	0.00092	5.7E-04	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	391.09	22.93	351.90	461.58	3.8101
IC10	537.71	61.05	370.47	681.85	-0.1189
IC15	640.13	28.23	521.00	717.27	-0.7112
IC20	693.71	27.30	618.48	786.72	0.2018
IC25	747.29	21.02	658.93	777.02	-0.9202
IC40	795.66	8.06	769.01	817.17	-0.2107
IC50	826.63	7.43	806.59	848.22	0.0238



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **121**

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.

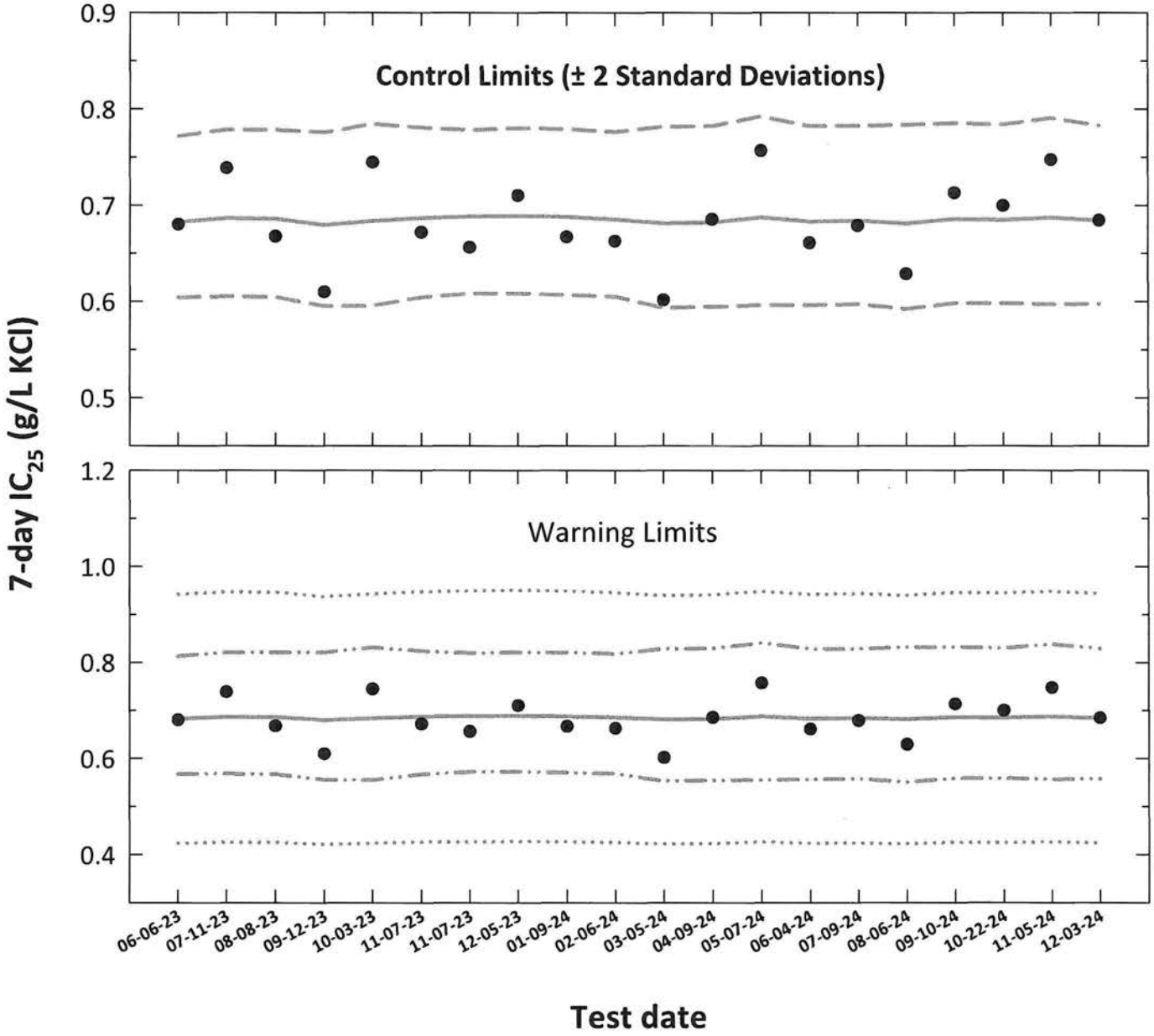
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	N	N	N	EM	EM	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.71	7.54	7.71	7.33	7.51	7.46
	Dissolved oxygen (mg/L)	7.7	7.7	7.7	7.4	7.7	7.3
	Conductivity (µmhos/cm)	298		308		312	
	Alkalinity (mg $CaCO_3$/L)	61				61	
	Hardness (mg $CaCO_3$/L)	92			N 1-12-24 N	92	
	Temperature (°C)	24.9	25.0	24.8	24.9	24.9	25.0
300 mg KCl/L	pH (S.U.)	7.92	7.54	7.84	7.39	7.55	7.41
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.5	7.8	7.4
	Conductivity (µmhos/cm)	833		847		868	
	Temperature (°C)	25.0	25.1	24.9	25.1	25.0	25.2
450 mg KCl/L	pH (S.U.)	7.92	7.59	7.84	7.43	7.59	7.39
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.6	7.8	6.6
	Conductivity (µmhos/cm)	1060		1080		1130	
	Temperature (°C)	25.0	25.3	24.9	25.1	25.0	25.2
600 mg KCl/L	pH (S.U.)	7.93	7.62	7.84	7.47	7.61	7.38
	Dissolved oxygen (mg/L)	7.8	7.7	7.9	7.5	7.8	6.4
	Conductivity (µmhos/cm)	1300		1300		1370	
	Temperature (°C)	25.0	25.0	24.9	25.0	25.0	24.9
750 mg KCl/L	pH (S.U.)	7.93	7.65	7.86	7.49	7.64	7.38
	Dissolved oxygen (mg/L)	7.8	7.6	7.9	7.5	7.8	6.4
	Conductivity (µmhos/cm)	1520		1530		1630	
	Temperature (°C)	24.8	25.0	24.8	25.2	25.0	24.9
900 mg KCl/L	pH (S.U.)	7.94	7.68	7.88	7.57	7.66	7.41
	Dissolved oxygen (mg/L)	7.8	7.6	8.0	7.5	7.8	6.4
	Conductivity (µmhos/cm)	1780		1900		1880	
	Temperature (°C)	24.8	25.0	24.8	24.9	25.0	24.9
1050 mg KCl/L	pH (S.U.)	7.95	7.69	7.89	7.57	7.68	7.44
	Dissolved oxygen (mg/L)	7.9	7.8	8.0	7.5	7.8	6.8
	Conductivity (µmhos/cm)	2060		2130		2150	
	Temperature (°C)	24.8	25.2	24.9	24.9	25.0	25.1
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **121**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	K	K	K	K	K	BM	BM	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.80	7.15	7.58	7.34	7.83	7.26	7.44	7.34
	Dissolved oxygen (mg/L)	7.7	7.5	7.9	7.7	7.9	7.5	7.9	7.4
	Conductivity (µmhos/cm)	296		293		296		316	
	Alkalinity (mg $CaCO_3$/L)			61					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	25.0	25.1	24.9	24.7	24.9	25.1	24.8	25.2
300 mg KCl/L	pH (S.U.)	7.90	7.25	7.72	7.31	7.91	7.32	7.53	7.31
	Dissolved oxygen (mg/L)	7.7	7.5	7.9	6.5	7.9	7.4	7.8	7.5
	Conductivity (µmhos/cm)	839		824		823		871	
	Temperature (°C)	25.1	24.9	25.0	24.9	25.0	24.7	24.9	25.0
450 mg KCl/L	pH (S.U.)	7.90	7.27	7.76	7.33	7.95	7.35	7.56	7.32
	Dissolved oxygen (mg/L)	7.7	6.7	7.9	6.3	7.9	7.5	7.9	7.2
	Conductivity (µmhos/cm)	1110		1080		1080		1140	
	Temperature (°C)	25.1	24.9	25.0	24.7	24.8	24.9	24.8	25.0
600 mg KCl/L	pH (S.U.)	7.91	7.34	7.78	7.33	7.96	7.40	7.59	7.35
	Dissolved oxygen (mg/L)	7.8	6.6	7.9	5.9	7.9	7.5	7.9	6.7
	Conductivity (µmhos/cm)	1330		1330		1340		1400	
	Temperature (°C)	25.1	25.2	25.0	24.7	24.8	24.8	24.8	25.3
750 mg KCl/L	pH (S.U.)	7.92	7.40	7.80	7.40	7.97	7.40	7.66	7.33
	Dissolved oxygen (mg/L)	7.9	6.6	7.8	5.8	7.9	7.5	8.0	6.7
	Conductivity (µmhos/cm)	1600		1580		1590		1670	
	Temperature (°C)	25.1	25.2	24.9	24.6	24.8	24.8	24.8	25.1
900 mg KCl/L	pH (S.U.)	7.92	7.38	7.81	7.48	7.98	7.46	7.65	7.34
	Dissolved oxygen (mg/L)	7.9	6.8	7.8	6.5	7.9	7.5	8.0	6.6
	Conductivity (µmhos/cm)	1860		1860		1830		1930	
	Temperature (°C)	25.1	25.0	24.9	24.8	24.8	25.0	24.8	25.1
1050 mg KCl/L	pH (S.U.)	7.92	7.49	7.84	7.53	8.00	7.49	7.67	7.40
	Dissolved oxygen (mg/L)	8.0	6.7	7.9	6.8	7.8	7.4	8.0	6.5
	Conductivity (µmhos/cm)	2110		2100 ~~2107~~		2080		2210	
	Temperature (°C)	25.2	25.2	24.9	24.6	24.9	24.8	24.9	25.1
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

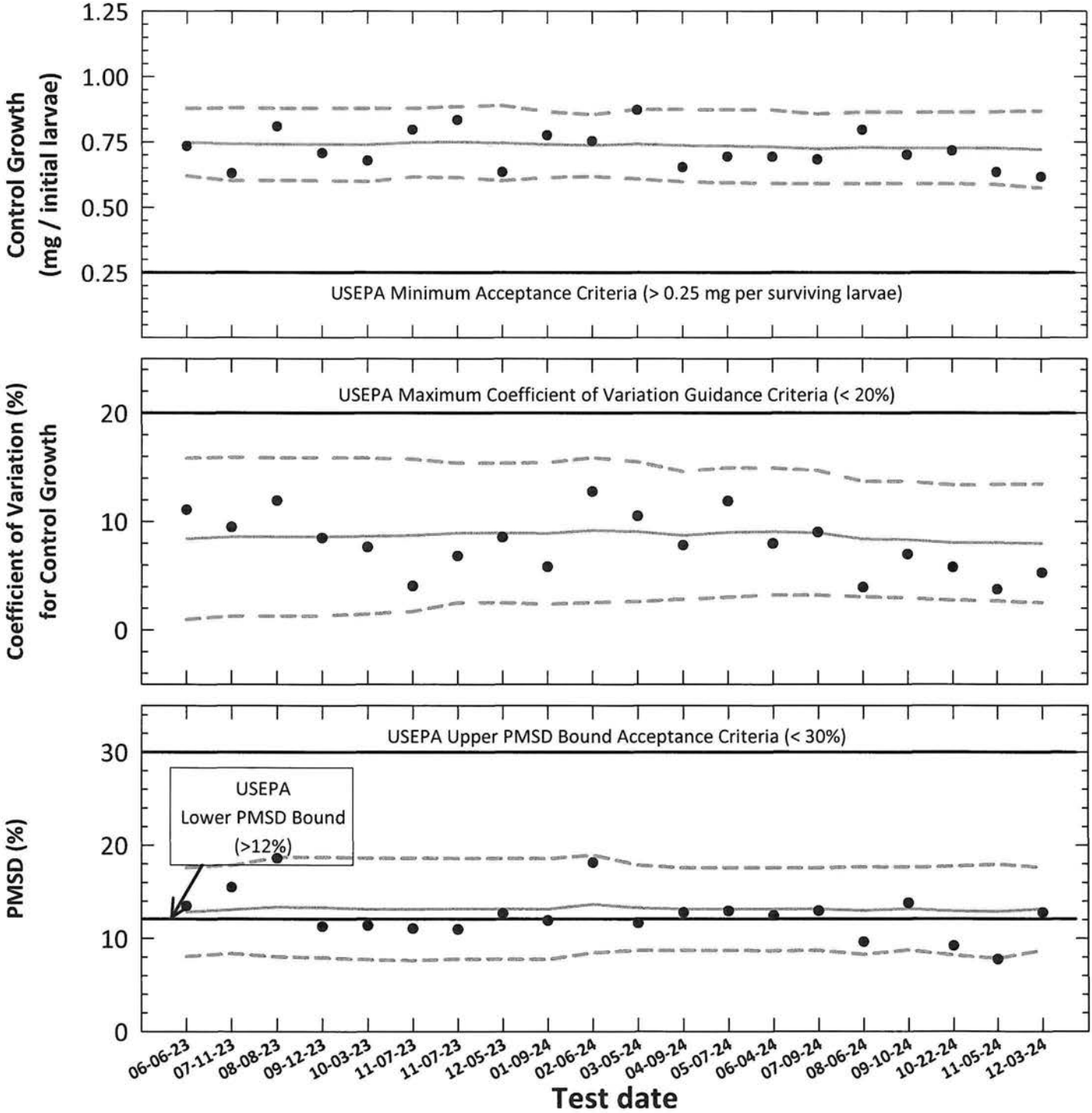
Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	06-06-23	**0.6800**	-0.1675	-0.1657	0.0266	0.6828	0.6041	0.7717	0.5676	0.8130	0.4233	0.9422
2	07-11-23	**0.7387**	-0.1315	-0.1632	0.0273	0.6868	0.6056	0.7789	0.5686	0.8208	0.4258	0.9478
3	08-08-23	**0.6677**	-0.1754	-0.1637	0.0274	0.6860	0.6045	0.7784	0.5673	0.8207	0.4253	0.9467
4	09-12-23	**0.6098**	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
5	10-03-23	**0.7447**	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436
6	11-07-23	**0.6718**	-0.1728	-0.1631	0.0278	0.6869	0.6045	0.7806	0.5669	0.8232	0.4259	0.9479
7	11-07-23	**0.6562**	-0.1830	-0.1622	0.0268	0.6883	0.6085	0.7786	0.5724	0.8194	0.4268	0.9499
8	12-05-23	**0.7102**	-0.1486	-0.1617	0.0269	0.6891	0.6088	0.7801	0.5725	0.8211	0.4273	0.9510
9	01-09-24	**0.6671**	-0.1758	-0.1624	0.0271	0.6880	0.6073	0.7795	0.5707	0.8209	0.4266	0.9495
10	02-06-24	**0.6628**	-0.1786	-0.1640	0.0270	0.6854	0.6052	0.7762	0.5684	0.8179	0.4250	0.9459
11	03-05-24	**0.6019**	-0.2205	-0.1666	0.0298	0.6814	0.5940	0.7817	0.5531	0.8286	0.4225	0.9404
12	04-09-24	**0.6855**	-0.1640	-0.1660	0.0298	0.6823	0.5949	0.7825	0.5542	0.8292	0.4230	0.9416
13	05-07-24	**0.7569**	-0.1210	-0.1627	0.0309	0.6876	0.5965	0.7927	0.5551	0.8404	0.4263	0.9489
14	06-04-24	**0.6610**	-0.1798	-0.1656	0.0295	0.6830	0.5963	0.7823	0.5561	0.8283	0.4234	0.9425
15	07-09-24	**0.6789**	-0.1682	-0.1650	0.0293	0.6839	0.5976	0.7826	0.5577	0.8283	0.4240	0.9438
16	08-06-24	**0.6287**	-0.2015	-0.1667	0.0304	0.6813	0.5922	0.7837	0.5506	0.8316	0.4224	0.9402
17	09-10-24	**0.7128**	-0.1470	-0.1640	0.0295	0.6856	0.5983	0.7855	0.5583	0.8313	0.4250	0.9461
18	10-22-24	**0.6997**	-0.1551	-0.1644	0.0294	0.6849	0.5983	0.7841	0.5585	0.8297	0.4247	0.9452
19	11-05-24	**0.7473**	-0.1265	-0.1629	0.0305	0.6872	0.5971	0.7908	0.5561	0.8380	0.4261	0.9483
20	12-03-24	**0.6844**	-0.1647	-0.1648	0.0293	0.6842	0.5979	0.7829	0.5580	0.8285	0.4242	0.9442

Note:
7-day IC_{25} = 25% inhibition concentration. An estimation of the potassium chloride concentration that would cause a 25% reduction in *Pimephales* growth (calculated using ToxCalc).
CT = Central tendency of the IC_{25} values.
S = Standard deviation of the IC_{25} values.
Control Limits = Mean logarithmic IC_{25} ± 2 standard deviations converted to anti-logarithmic values.
Warning Limits = Mean logarithmic IC_{25} ± 2CV or $S_{A.75}$ converted to anti-logarithmic values.
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).
CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Growth (mg/initial larvae)			Control Growth CV (%)			Test PMSD (%)		
		Control Survival (%)	Control Growth Mean (mg/initial larvae)	Control Growth CV (%)	Test MSD	Test PMSD (%)	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S	CT	95% Confidence Interval CT - 2S	95% Confidence Interval CT + 2S
1	06-06-23	100	0.734	11.1	0.0988	13.5	0.749	0.619	0.878	8.4	1.0	15.8	12.8	8.0	17.6
2	07-11-23	100	0.630	9.5	0.0976	15.5	0.742	0.603	0.882	8.6	1.3	15.9	13.1	8.4	17.8
3	08-08-23	100	0.809	11.9	0.1504	18.6	0.741	0.603	0.879	8.6	1.3	15.9	13.4	8.0	18.7
4	09-12-23	100	0.707	8.5	0.0796	11.3	0.740	0.602	0.879	8.6	1.3	15.9	13.3	7.9	18.7
5	10-03-23	100	0.678	7.7	0.0772	11.4	0.739	0.599	0.880	8.7	1.5	15.9	13.2	7.7	18.6
6	11-07-23	100	0.797	4.0	0.0880	11.0	0.748	0.616	0.880	8.7	1.7	15.8	13.1	7.6	18.6
7	11-07-23	100	0.833	6.8	0.0913	11.0	0.750	0.614	0.886	8.9	2.5	15.4	13.2	7.8	18.6
8	12-05-23	100	0.635	8.6	0.0805	12.7	0.746	0.602	0.890	9.0	2.5	15.4	13.2	7.8	18.6
9	01-09-24	100	0.776	5.8	0.0923	11.9	0.740	0.614	0.866	8.9	2.4	15.4	13.1	7.7	18.6
10	02-06-24	100	0.753	12.8	0.1364	18.1	0.736	0.618	0.855	9.2	2.5	15.9	13.7	8.4	18.9
11	03-05-24	100	0.873	10.5	0.1016	11.6	0.742	0.609	0.876	9.1	2.6	15.5	13.3	8.7	17.8
12	04-09-24	100	0.653	7.8	0.0834	12.8	0.736	0.598	0.874	8.7	2.8	14.6	13.1	8.7	17.6
13	05-07-24	100	0.693	11.9	0.0896	12.9	0.734	0.595	0.874	9.0	3.0	15.0	13.1	8.7	17.6
14	06-04-24	100	0.693	8.0	0.0862	12.4	0.732	0.591	0.872	9.1	3.2	14.9	13.1	8.7	17.6
15	07-09-24	100	0.683	9.0	0.0883	12.9	0.724	0.591	0.857	9.0	3.2	14.7	13.1	8.7	17.6
16	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	8.3	17.7
17	09-10-24	100	0.701	7.0	0.0964	13.8	0.728	0.591	0.865	8.3	2.9	13.7	13.2	8.7	17.6
18	10-22-24	100	0.717	5.8	0.0659	9.2	0.727	0.590	0.864	8.1	2.7	13.4	12.9	8.2	17.7
19	11-05-24	100	0.635	3.7	0.0491	7.7	0.727	0.588	0.866	8.0	2.7	13.4	12.9	7.8	17.9
20	12-03-24	100	0.615	5.3	0.0785	12.8	0.721	0.573	0.868	8.0	2.5	13.5	13.1	8.7	17.6

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: **122**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2334				
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:	11-27-24	*Artemia* CHM number:	CHM1294
Hatch dates and times:	12-02-24 1452 TO 12-03-24 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 7.68 S.U. Temperature = 24.0 °C	Date / Time in oven:	12-10-24 0530
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	12-11-24 0530
		*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	12-03-24	0505	JH	1340	JH	0715	JH	11-27-24 A
1	12-04-24	0500	JH	1415	JH	0700	JH	↓
2	12-05-24	0500	JH	1310	JH	0700	JH	11-27-24 C
3	12-06-24	0500	JH	1100	JH	0720	JH	↓
4	12-07-24	0500	JH	1130	JH	0900	JH	12-05-24
5	12-08-24	0500	JH	1100	JH	0700	JH	↓
6	12-09-24	0500	JH	1210	JH	0700	JH	↓
7	12-10-24					0516	JH	

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 G-2021	YSI Model 52CE	08A100271
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	13066465

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% Mortality:	0%	≤ 20%	**7-day LC_{50}** (mg/L KCl)	600.0
Average weight per initial larvae:	0.615		**NOEC** (mg/L KCl)	450
Average weight per surviving larvae:	0.615	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	600
			ChV (mg/L KCl)	519.6
			IC_{25} (mg/L KCl)	684.4

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **122**

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: JP light pink Analyst: JP Date: 11-25-24	11.86	12.82	11.09	14.69	11.75	14.41	12.23	13.47	12.58	13.82	13.58	14.05
*B = Pan + Larvae weight (mg) Analyst: JP Date: 12-12-24	18.15	18.78	16.91	21.23	18.49	21.10	19.77	20.34	19.10	20.84	19.33	21.47
C = Larvae weight (mg) = B – A Analyst: JP	6.29	5.96	5.82	6.54	6.74	6.69	7.54	6.87	6.52	7.02	5.75	7.42
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JP	0.629	0.596	0.582	0.654	0.674	0.669	0.754	0.687	0.652	0.702	0.575	0.742
Average weight per initial number of larvae (mg)	0.615				0.696				0.668			
Percent reduction from control (%)							-13.17.				-8.57.	

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **122**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	9 1d	10	10	7 3d	6 4d	7 3d	6 4d
3	10	10	10	10	10	9	10	9 1d	6 1d	6	7	5
4	10	10	10	10	10	9	10	9	6	6	7	5
5	9 1d	9 1d	9 1d	9 1d	10	9	9 1d	9	6	6	6 1d	5
6	9	9	9	9	9 1d	9	8 1d	8 1d	4 2d	4 2d	5 1d	5
7	9	9	8 1d	8 1d	6 3d	7 2d	7 1d	7 1d	3 1d	2 2d	3 2d	3 2d
*A = Pan weight (mg) Tray color code: light pink Analyst: JP Date: 11-25-24	14.39	13.01	14.41	13.83	15.20	15.24	15.77	13.19	14.65	13.61	15.36	13.39
*B = Pan + Larvae weight (mg) Analyst: JH Date: 12-12-24	21.08	18.76	19.06	18.94	19.32	19.69	20.53	17.77	16.48	14.89	17.09	15.13
C = Larvae weight (mg) = B – A Analyst: JH	6.70	5.75	4.65	5.11	4.12	4.45	4.76	4.58	1.83	1.28	1.73	1.74
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.670	0.575	0.465	0.511	0.412	0.445	0.476	0.458	0.183	0.128	0.173	0.174
Average weight per initial number of larvae (mg)	0.555				0.448				0.165			
Percent reduction from control (%)		9.87.				27.27.				73.37.		

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **122**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	10	9 d	9 d	9 d
2	5 sd	5 4d	5 4d	7 2d
3	4 d	4 d	5	6 d
4	4	4	4 d	4 d
5	3 d	3 d	3 d	4
6	2 d	2 d	2 d	2 2d
7	2	1 d	1 d	1 d
*A = Pan weight (mg) Tray color code: light pink Analyst: JP Date: 11-25-24	12.26	12.93	15.42	13.79
*B = Pan + Larvae weight (mg) Analyst: JH Date: 12-12-24	13.48	13.46	16.28	14.43
C = Larvae weight (mg) = B – A Analyst: JH	1.22	0.53	0.86	0.64
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JH	0.122	0.053	0.086	0.064
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.081		86.87	

*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: 122

Test dates: December 03-10, 2024

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	11.86	18.15	6.29	0.629	0.615	5.3	0.629	100.0	0.615	5.3	Not applicable
	B	10	10	12.82	18.78	5.96	0.596			0.596				
	C	10	10	11.09	16.91	5.82	0.582			0.582				
	D	10	10	14.69	21.23	6.54	0.654			0.654				
300	E	10	10	11.75	18.49	6.74	0.674	0.696	5.7	0.674	100.0	0.696	5.7	-13.1
	F	10	10	14.41	21.10	6.69	0.669			0.669				
	G	10	10	12.23	19.77	7.54	0.754			0.754				
	H	10	10	13.47	20.34	6.87	0.687			0.687				
450	I	10	10	12.58	19.10	6.52	0.652	0.668	10.8	0.652	100.0	0.668	10.8	-8.5
	J	10	10	13.82	20.84	7.02	0.702			0.702				
	K	10	10	13.58	19.33	5.75	0.575			0.575				
	L	10	10	14.05	21.47	7.42	0.742			0.742				
600	M	10	9	14.38	21.08	6.70	0.744	0.651	10.5	0.670	85.0	0.555	16.0	9.8
	N	10	9	13.01	18.76	5.75	0.639			0.575				
	O	10	8	14.41	19.06	4.65	0.581			0.465				
	P	10	8	13.83	18.94	5.11	0.639			0.511				
750	Q	10	6	15.20	19.32	4.12	0.687	0.664	3.5	0.412	67.5	0.448	6.0	27.2
	R	10	7	15.24	19.69	4.45	0.636			0.445				
	S	10	7	15.77	20.53	4.76	0.680			0.476				
	T	10	7	13.19	17.77	4.58	0.654			0.458				
900	U	10	3	14.65	16.48	1.83	0.610	0.602	4.9	0.183	27.5	0.165	15.0	73.3
	V	10	2	13.61	14.89	1.28	0.640			0.128				
	W	10	3	15.36	17.09	1.73	0.577			0.173				
	X	10	3	13.39	15.13	1.74	0.580			0.174				
1050	Y	10	2	12.26	13.48	1.22	0.610	0.660	21.4	0.122	12.5	0.081	37.5	86.8
	Z	10	1	12.93	13.46	0.53	0.530			0.053				
	AA	10	1	15.42	16.28	0.86	0.860			0.086				
	BB	10	1	13.79	14.43	0.64	0.640			0.064				

Dunnett's MSD value: 0.0785 MSD = Minimum Significant Difference

PMSD: 12.8 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	12/3/2024	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	12/10/2024	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	PP-Pimephales promelas
Comments:					

Conc-mg/L	1	2	3	4
D-Control	1.0000	1.0000	1.0000	1.0000
300	1.0000	1.0000	1.0000	1.0000
450	1.0000	1.0000	1.0000	1.0000
600	0.9000	0.9000	0.8000	0.8000
750	0.6000	0.7000	0.7000	0.7000
900	0.3000	0.2000	0.3000	0.3000
1050	0.2000	0.1000	0.1000	0.1000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
*600	0.8500	0.8500	1.1781	1.1071	1.2490	6.954	4	10.00	10.00	6	40
*750	0.6750	0.6750	0.9649	0.8861	0.9912	5.445	4	10.00	10.00	13	40
*900	0.2750	0.2750	0.5506	0.4636	0.5796	10.532	4	10.00	10.00	29	40
*1050	0.1250	0.1250	0.3572	0.3218	0.4636	19.861	4	10.00	10.00	35	40

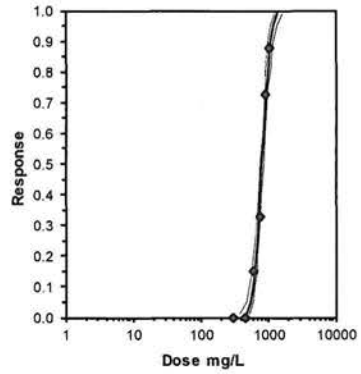
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91923	0.896	0.02303	0.55288
Equality of variance cannot be confirmed				

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

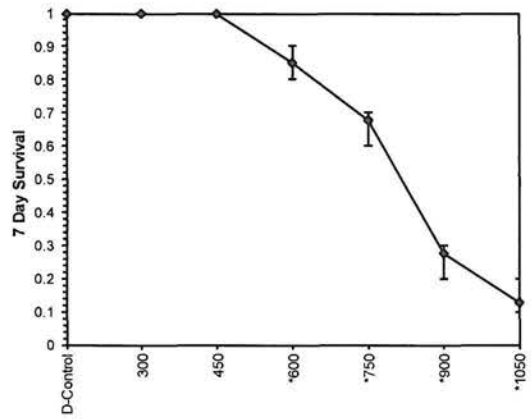
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	9.86276	1.23648	7.43926	12.2863	0	1.90087	9.48773	0.75399	2.90308	0.10139	3
Intercept	-23.632	3.59076	-30.67	-16.594							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	464.734	385.813	522.128
EC05	3.355	544.881	474.722	595.375
EC10	3.718	593.113	529.559	639.324
EC15	3.964	628.044	569.583	671.395
EC20	4.158	657.268	603.064	698.579
EC25	4.326	683.421	632.853	723.344
EC40	4.747	754.03	711.031	793.684
EC50	5.000	799.974	758.849	843.445
EC60	5.253	848.718	806.299	900.31
EC75	5.674	936.405	884.539	1011.72
EC80	5.842	973.664	915.854	1061.74
EC85	6.036	1018.97	952.898	1124.2
EC90	6.282	1078.98	1000.66	1209.21
EC95	6.645	1174.49	1074.5	1348.93
EC99	7.326	1377.04	1225.2	1659.83



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	12/3/2024	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	12/10/2024	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	0.6290	0.5960	0.5820	0.6540
300	0.6740	0.6690	0.7540	0.6870
450	0.6520	0.7020	0.5750	0.7420
600	0.6700	0.5750	0.4650	0.5110
750	0.4120	0.4450	0.4760	0.4580
900	0.1830	0.1280	0.1730	0.1740
1050	0.1220	0.0530	0.0860	0.0640

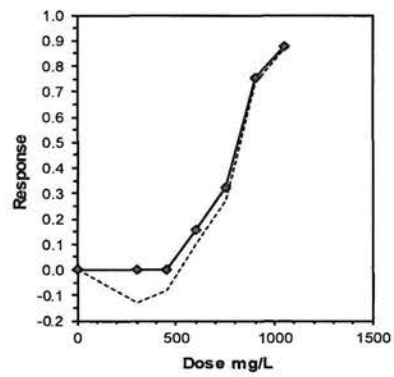
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.6153	1.0000	0.6153	0.5820	0.6540	5.281	4				0.6597	1.0000
300	0.6960	1.1312	0.6960	0.6690	0.7540	5.661	4	-2.241	2.180	0.0785	0.6597	1.0000
450	0.6678	1.0853	0.6678	0.5750	0.7420	10.777	4	-1.457	2.180	0.0785	0.6597	1.0000
600	0.5553	0.9025	0.5553	0.4650	0.6700	15.994	4				0.5553	0.8417
750	0.4478	0.7278	0.4478	0.4120	0.4760	6.033	4				0.4478	0.6788
900	0.1645	0.2674	0.1645	0.1280	0.1830	15.043	4				0.1645	0.2494
1050	0.0813	0.1321	0.0813	0.0530	0.1220	37.458	4				0.0813	0.1232

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95509	0.805	-0.191	0.21399
Bartlett's Test indicates equal variances (p = 0.39)	1.88895	9.21035		

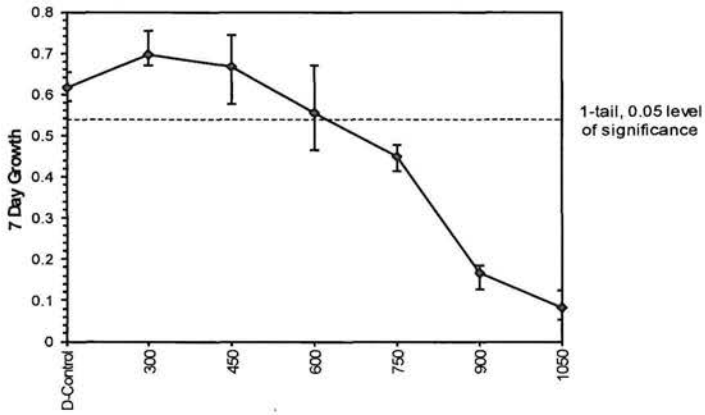
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	450	>450			0.07854	0.12765	0.00672	0.0026	0.12949	2, 9
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	497.38	39.90	300.46	589.87	-0.7983
IC10	544.76	41.24	390.92	663.08	-0.1745
IC15	592.15	37.01	483.24	688.87	-0.2200
IC20	638.40	36.10	506.82	710.02	-0.6153
IC25	684.42	34.06	528.07	740.01	-1.1981
IC40	777.51	6.19	754.90	793.95	-0.3261
IC50	812.44	5.10	792.35	825.62	-0.2951



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **122**

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	M	EM	EM	EM	EM	EM
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.06	7.41	7.47	7.22	7.35	7.27
	Dissolved oxygen (mg/L)	7.6	7.6	7.9	7.6	7.6	7.5
	Conductivity (µmhos/cm)	292		318		317	
	Alkalinity (mg $CaCO_3$/L)	59				57	
	Hardness (mg $CaCO_3$/L)	88				88	
	Temperature (°C)	24.8	25.0	24.7	25.0	24.8	25.0
300 mg KCl/L	pH (S.U.)	7.07	7.49	7.62	7.32	7.51	7.33
	Dissolved oxygen (mg/L)	7.8	7.6	7.8	7.6	7.7	7.6
	Conductivity (µmhos/cm)	813		874		865	
	Temperature (°C)	24.9	24.9	24.7	25.0	24.9	24.7
450 mg KCl/L	pH (S.U.)	7.07	7.49	7.64	7.34	7.52	7.37
	Dissolved oxygen (mg/L)	7.8	7.6	7.7	7.5	7.7	7.6
	Conductivity (µmhos/cm)	1050		1130		1120	
	Temperature (°C)	24.9	25.1	24.8	25.2	24.9	24.8
600 mg KCl/L	pH (S.U.)	7.07	7.51	7.65	7.33	7.53	7.41
	Dissolved oxygen (mg/L)	7.9	7.6	7.6	7.5	7.7	7.6
	Conductivity (µmhos/cm)	1290		1390		1380	
	Temperature (°C)	24.9	25.1	24.7	24.9	25.0	24.8
750 mg KCl/L	pH (S.U.)	7.07	7.52	7.65	7.35	7.54	7.41
	Dissolved oxygen (mg/L)	8.0	7.6	7.6	7.6	7.7	7.6
	Conductivity (µmhos/cm)	1540		1660		1620	
	Temperature (°C)	24.9	24.9	24.8	24.9	25.0	24.6
900 mg KCl/L	pH (S.U.)	7.07	7.55	7.66	7.37	7.55	7.43
	Dissolved oxygen (mg/L)	8.0	7.6	7.6	7.6	7.7	7.6
	Conductivity (µmhos/cm)	1900		1930		1900	
	Temperature (°C)	24.9	24.9	24.8	24.9	24.8	24.6
1050 mg KCl/L	pH (S.U.)	7.07	7.54	7.67	7.41	7.56	7.43
	Dissolved oxygen (mg/L)	8.1	7.6	7.6	7.6	7.7	7.6
	Conductivity (µmhos/cm)	2050		2210		2170	
	Temperature (°C)	25.0	25.0	24.8	25.1	24.8	24.6
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **122**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	EM	N	N	N	N	EM	EM	EM
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	7.51	7.53	7.81	7.37	7.86	7.27	7.27	7.28
	Dissolved oxygen (mg/L)	7.6	6.8	7.7	6.8	7.9	7.0	7.7	7.0
	Conductivity (µmhos/cm)	315		310		298		310	
	Alkalinity (mg $CaCO_3$/L)			59					
	Hardness (mg $CaCO_3$/L)			84					
	Temperature (°C)	24.9	24.9	24.7	24.9	24.8	25.1	24.8	24.7
300 mg KCl/L	pH (S.U.)	7.65	7.44	7.84	7.39	7.88	7.43	7.66	7.76
	Dissolved oxygen (mg/L)	7.7	6.8	7.7	6.9	7.9	7.2	7.9	6.9
	Conductivity (µmhos/cm)	853		824		845		855	
	Temperature (°C)	24.8	24.7	24.6	24.6	24.9	24.9	24.7	24.9
450 mg KCl/L	pH (S.U.)	7.65	7.43	7.85	7.44	7.88	7.42	7.66	7.77
	Dissolved oxygen (mg/L)	7.7	6.8	7.8	6.6	7.9	7.2	7.9	6.9
	Conductivity (µmhos/cm)	1120		1070		1100		1110	
	Temperature (°C)	24.8	24.9	24.6	24.9	24.9	24.9	24.7	24.9
600 mg KCl/L	pH (S.U.)	7.66	7.43	7.85	7.47	7.90	7.44	7.68	7.76
	Dissolved oxygen (mg/L)	7.7	6.7	7.8	6.5	7.9	7.1	7.9	6.9
	Conductivity (µmhos/cm)	1380		1330		1360		1370	
	Temperature (°C)	24.8	24.9	24.6	25.0	24.9	25.1	24.8	24.6
750 mg KCl/L	pH (S.U.)	7.67	7.47	7.86	7.47	7.92	7.45	7.69	7.72
	Dissolved oxygen (mg/L)	7.7	6.8	7.9	6.6	8.0	7.2	7.9	7.0
	Conductivity (µmhos/cm)	1640		1600		1600		1620	
	Temperature (°C)	24.9	25.0	24.6	25.0	25.0	25.1	24.8	24.6
900 mg KCl/L	pH (S.U.)	7.69	7.57	7.86	7.51	7.92	7.50	7.70	7.76
	Dissolved oxygen (mg/L)	7.8	6.9	8.0	6.5	8.0	7.2	7.9	7.0
	Conductivity (µmhos/cm)	1910		1890		1920		1880	
	Temperature (°C)	24.9	24.8	24.7	24.7	25.0	24.8	24.8	24.6
1050 mg KCl/L	pH (S.U.)	7.70	7.45	7.88	7.52	7.94	7.46	7.71	7.74
	Dissolved oxygen (mg/L)	7.8	6.4	8.0	6.9	8.0	7.2	7.9	7.0
	Conductivity (µmhos/cm)	2190		2090		2090		2140	
	Temperature (°C)	24.9	24.8	24.7	24.7	25.0	25.0	24.9	24.6
		Initial	Final	Initial	Final	Initial	Final	Initial	Final