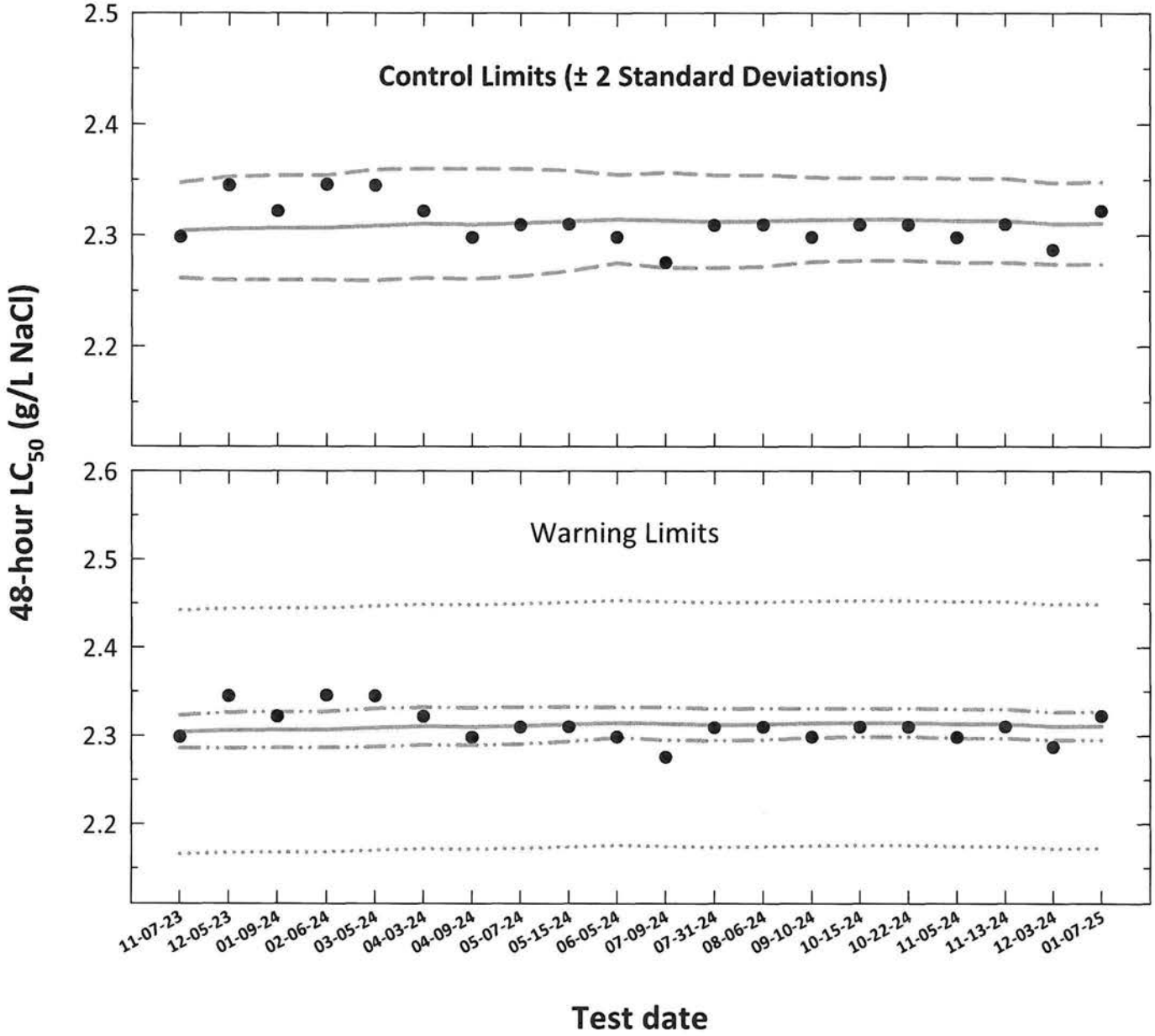
Ceriodaphnia dubia
Acute Reference Toxicant Control Chart
Source: In-house Culture



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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	48-hour LC_{50} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			48-hour LC_{50}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	01-07-25	2.3220	0.3659	0.3638	0.0035	2.3110	2.2743	2.3482	2.2951	2.3271	2.1723	2.4496

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

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 # 421

Dilution Preparation:

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

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

Stock solution INSS #: 2342

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	BM	BM	BM
Control, MHSW	pH (S.U.)	8.00	8.03	8.00
	Dissolved oxygen (mg/L)	7.6	7.6	7.6
	Conductivity (µmhos/cm)	316		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	87		
	Temperature (°C)	24.8	25.1	25.2
1750 mg/L	pH (S.U.)	7.98	7.96	7.94
	Dissolved oxygen (mg/L)	7.7	7.6	7.6
	Conductivity (µmhos/cm)	3560		
	Temperature (°C)	24.9	25.0	25.0
2000 mg/L	pH (S.U.)	8.00	7.95	7.95
	Dissolved oxygen (mg/L)	7.7	7.6	7.6
	Conductivity (µmhos/cm)	3470		
	Temperature (°C)	25.0	25.3	25.3
2250 mg/L	pH (S.U.)	8.01	7.97	7.96
	Dissolved oxygen (mg/L)	7.7	7.6	7.6
	Conductivity (µmhos/cm)	4350		
	Temperature (°C)	25.0	25.1	25.3
2500 mg/L	pH (S.U.)	8.02	7.96	7.95
	Dissolved oxygen (mg/L)	7.7	7.6	7.5
	Conductivity (µmhos/cm)	4790		
	Temperature (°C)	24.9	25.1	25.1
2750 mg/L	pH (S.U.)	8.02	7.96	7.95
	Dissolved oxygen (mg/L)	7.7	7.6	7.6
	Conductivity (µmhos/cm)	5240		
	Temperature (°C)	24.9	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	13066468S

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 # 421

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	01-07-25	* 0515	JI	0742	JI	2B2	BWC	01-07-25B
24	01-08-25			0740	JI			
48 Termination	01-09-25			0740	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
Source (organisms were pooled):	12-31-24 D
Age:	< 24-hours old
Date and time organisms were born between:	01-06-25 1440 TO 01-07-25 0515
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.95
	Temperature (°C): 24.9

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

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	5	5	4d	5	5	5	4d	3d	4d	2d
48 Termination	3d	4d	2d	4d	1d	1d	1d	0d	0d	0d	0d	0d
Mean Survival	65%				15%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2252.7
Upper 95% confidence limit (mg NaCl/L)	2388.9
48-hour LC_{50} (mg NaCl/L)	2322.0

Comments:

Test Reviewed by: JI

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	1/7/2025	Test ID:	CdNaCIAC	Sample ID:	REF-Ref Toxicant
End Date:	1/9/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

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

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.6500	0.6500	0.9463	0.6847	1.1071	21.467	4	10.00	10.00	7	20
*2500	0.1500	0.1500	0.4041	0.2255	0.4636	29.464	4	10.00	10.00	17	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

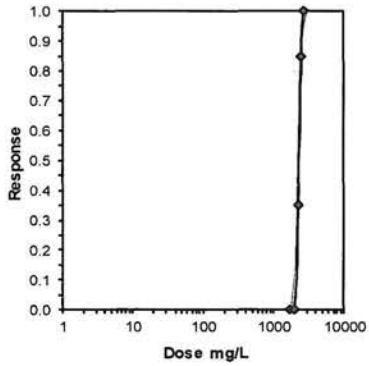
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.7885	0.868	-1.0601	3.14235
Equality of variance cannot be confirmed				

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

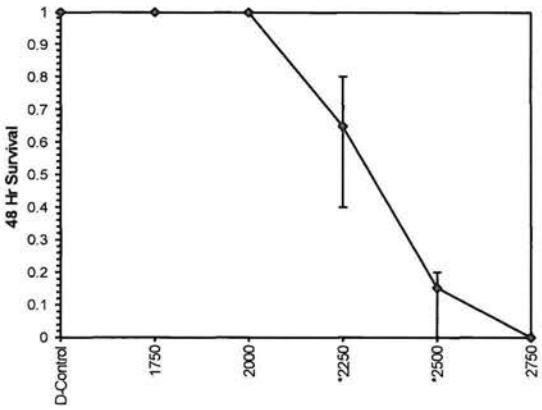
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	36.1735	7.14843	22.1625	50.1844	0	0.55322	7.81472	0.90705	3.36585	0.02764	4
Intercept	-116.75	24.0777	-163.95	-69.562							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	2002.37	1806.78	2102.24
EC05	3.355	2091.14	1935.4	2173.44
EC10	3.718	2140.06	2006.38	2213.79
EC15	3.964	2173.72	2054.84	2242.41
EC20	4.158	2200.84	2093.43	2266.25
EC25	4.326	2224.38	2126.38	2287.69
EC40	4.747	2284.82	2207.14	2347.48
EC50	5.000	2321.96	2252.74	2388.92
EC60	5.253	2359.71	2295.14	2435.47
EC75	5.674	2423.82	2358.62	2524.23
EC80	5.842	2449.75	2381.89	2562.92
EC85	6.036	2480.31	2408.08	2610.11
EC90	6.282	2519.32	2440.02	2672.27
EC95	6.645	2578.26	2486.11	2769.4
EC99	7.326	2692.56	2571.1	2965.63

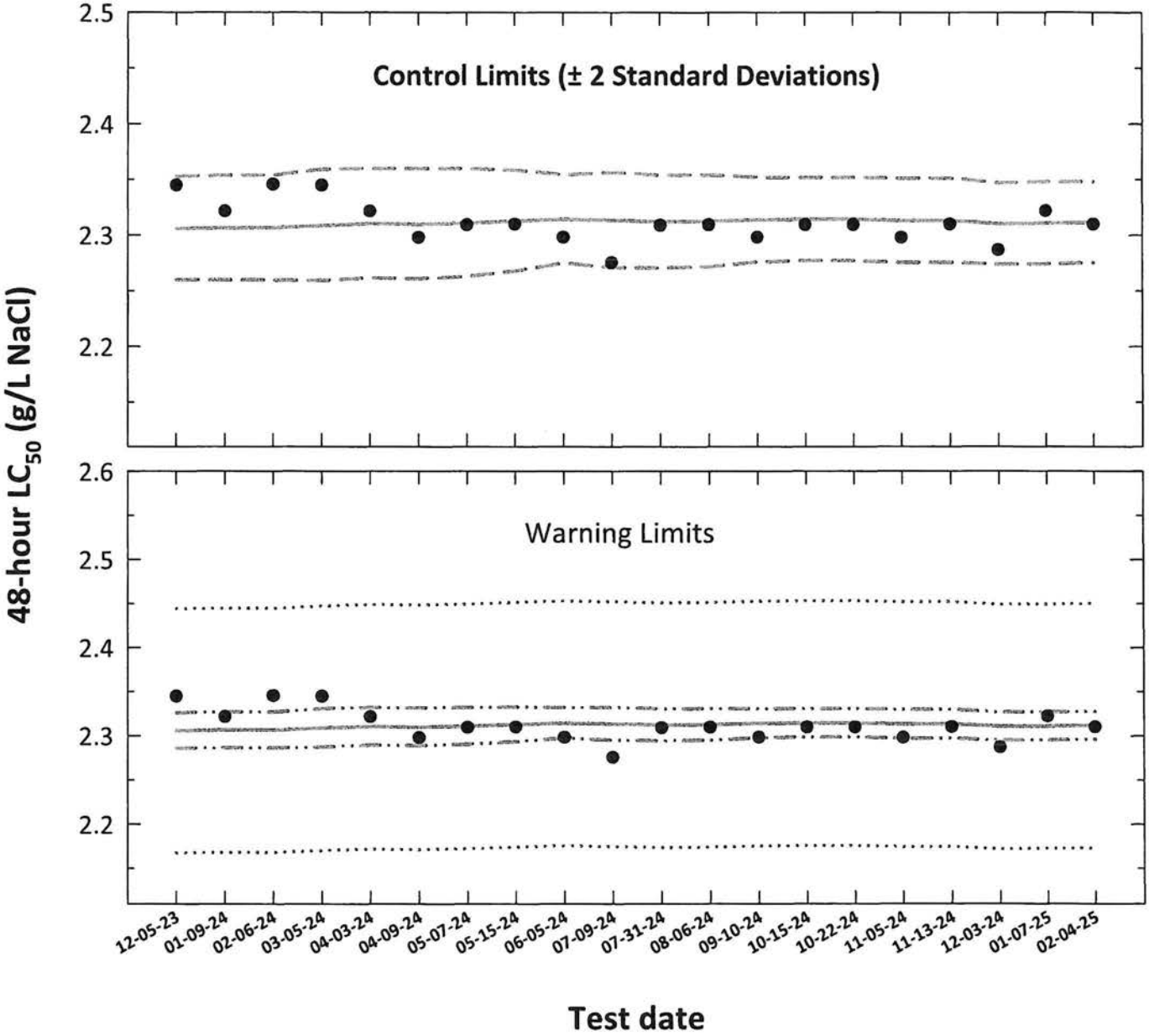


Dose-Response Plot



Environmental Testing Solutions, Inc.

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



- ● **48-hour LC_{50}** = median lethal concentration. An estimation of the sodium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic LC_{50} converted to anti-logarithmic values)
- **Control Limits** (mean logarithmic LC_{50} ± 2 standard deviations converted to anti-logarithmic values)
- **Laboratory Warning Limits** (mean logarithmic LC_{50} ± 2 coefficent of variations converted to anti-logarithmic values)
- ..... **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.10}$ 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	01-07-25	2.3220	0.3659	0.3638	0.0035	2.3110	2.2743	2.3482	2.2951	2.3271	2.1723	2.4496
20	02-04-25	2.3099	0.3636	0.3639	0.0034	2.3116	2.2754	2.3483	2.2959	2.3275	2.1729	2.4503

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.

Reviewed and Approved 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 # 422

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

Stock solution INSS #: 2342

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	N	Bm	Bm
Control, MHSW	pH (S.U.) 020423	~~3.04~~ 7.77	7.77	7.82
	Dissolved oxygen (mg/L)	8.05 3.0	7.7	7.7
	Conductivity (µmhos/cm)	300		
	Alkalinity (mg/L $CaCO_3$)	59		
	Hardness (mg/L $CaCO_3$)	90		
	Temperature (°C)	24.9	25.3	25.1
1750 mg/L	pH (S.U.)	7.99	7.75	7.82
	Dissolved oxygen (mg/L)	7.9	7.8	7.8
	Conductivity (µmhos/cm)	3370		
	Temperature (°C)	25.0	25.2	24.9
2000 mg/L	pH (S.U.)	7.99	7.76	7.83
	Dissolved oxygen (mg/L)	7.9	7.8	7.8
	Conductivity (µmhos/cm)	3730		
	Temperature (°C)	25.0	25.2	24.9
2250 mg/L	pH (S.U.)	7.97	7.76	7.82
	Dissolved oxygen (mg/L)	8.0	7.7	7.8
	Conductivity (µmhos/cm)	4260		
	Temperature (°C)	24.9	25.2	25.2
2500 mg/L	pH (S.U.)	7.80	7.76	7.83
	Dissolved oxygen (mg/L)	8.0	7.8	7.8
	Conductivity (µmhos/cm)	4610		
	Temperature (°C)	24.9	25.2	24.9
2750 mg/L	pH (S.U.)	7.99	7.76	7.83
	Dissolved oxygen (mg/L)	8.0	7.8	7.8
	Conductivity (µmhos/cm)	5170		
	Temperature (°C)	24.9	25.4	24.9

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 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	1306646

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	2/4/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	2/6/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:					

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

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4			0	20
1750	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
2000	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	4	18.00	10.00	0	20
*2250	0.5500	0.5500	0.8407	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

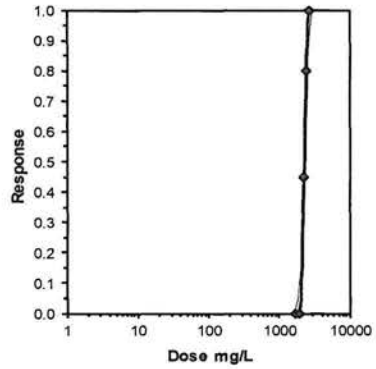
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.74905	0.868	0.45087	2.42089
Equality of variance cannot be confirmed				

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

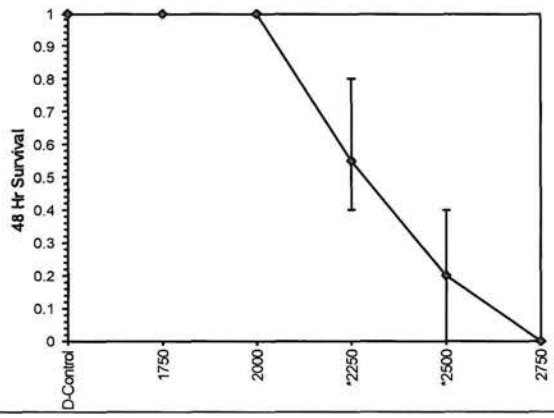
Maximum Likelihood-Probit

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											

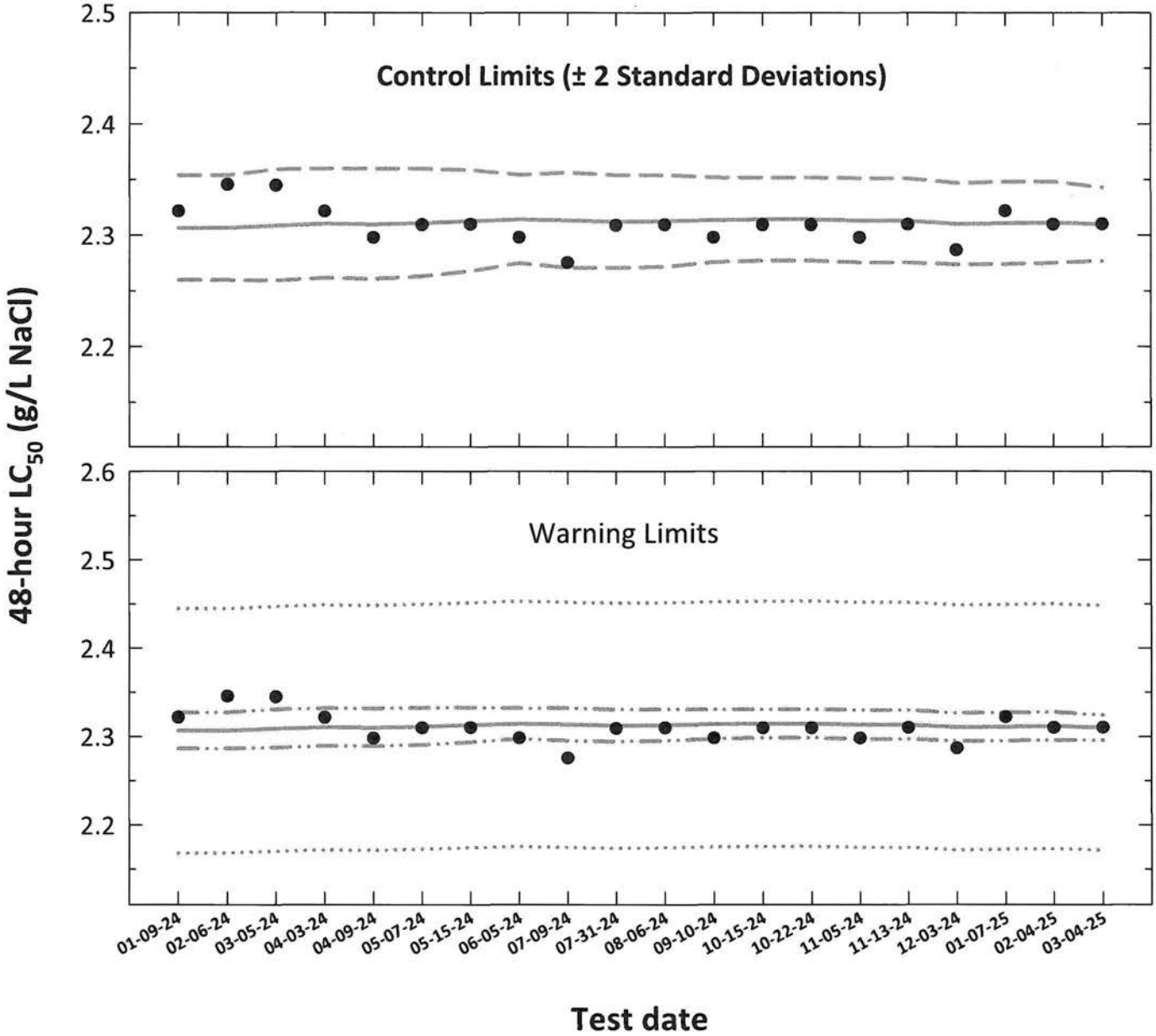
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1948.47	1752	2054.85
EC05	3.355	2048.07	1890.23	2136.13
EC10	3.718	2103.23	1966.98	2182.25
EC15	3.964	2141.28	2019.59	2214.95
EC20	4.158	2172.02	2061.61	2242.15
EC25	4.326	2198.74	2097.59	2266.57
EC40	4.747	2267.53	2186.32	2334.36
EC50	5.000	2309.94	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	**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
2	**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
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**01-07-25**	**2.3220**	0.3659	0.3638	0.0035	2.3110	2.2743	2.3482	2.2951	2.3271	2.1723	2.4496
19	**02-04-25**	**2.3099**	0.3636	0.3639	0.0034	2.3116	2.2754	2.3483	2.2959	2.3275	2.1729	2.4503
20	**03-04-25**	**2.3102**	0.3636	0.3636	0.0031	2.3098	2.2771	2.3431	2.2957	2.3242	2.1713	2.4484

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 Spencer

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 # 423

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

Stock solution INSS #: 2356

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	Bm	Bm	Bm
Control, MHSW	pH (S.U.)	7.96	7.90	7.92
	Dissolved oxygen (mg/L)	8.1	7.8	7.9
	Conductivity (µmhos/cm)	313		
	Alkalinity (mg/L $CaCO_3$)	58		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	25.0	25.2	25.3
1750 mg/L	pH (S.U.)	7.95	7.90	7.95
	Dissolved oxygen (mg/L)	8.2	7.9	8.0
	Conductivity (µmhos/cm)	3430		
	Temperature (°C)	25.1	25.0	25.1
2000 mg/L	pH (S.U.)	7.95	7.90	7.97
	Dissolved oxygen (mg/L)	8.2	7.8	7.9
	Conductivity (µmhos/cm)	3880		
	Temperature (°C)	25.1	25.3	25.1
2250 mg/L	pH (S.U.)	7.95	7.91	7.97
	Dissolved oxygen (mg/L)	8.2	7.9	7.9
	Conductivity (µmhos/cm)	4240		
	Temperature (°C)	25.0	25.1	24.9
2500 mg/L	pH (S.U.)	7.95	7.91	7.97
	Dissolved oxygen (mg/L)	8.2	7.9	7.9
	Conductivity (µmhos/cm)	4620		
	Temperature (°C)	25.0	25.1	24.9
2750 mg/L	pH (S.U.)	7.96	7.91	7.97
	Dissolved oxygen (mg/L)	8.2	7.9	7.9
	Conductivity (µmhos/cm)	5050		
	Temperature (°C)	25.2	25.0	24.9

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 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

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 # 423

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	03-04-25	* 0530 + 0800	JL	1138	JL	2F1	BLACK	02-26-25A
24	03-05-25			1141	JL			
48 Termination	03-06-25			1140	JL			

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

Test Organism Information:

Organism Source:	In-house Culture
Source (organisms were pooled):	02-25-25 D
Age:	< 24-hours old
Date and time organisms were born between:	03-03-25 1500 TO 03-04-25 0800
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.91
	Temperature (°C): 25.1

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

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

Hours	2250 mg/L				2500 mg/L				2750 mg/L			
	Replicate				Replicate				Replicate			
	M	N	O	P	Q	R	S	T	U	V	W	X
0 Initiation	5	5	5	5	5	5	5	5	5	5	5	5
24	S	S	S	S	4 1d	S	S	S	4 1d	4 1d	3 2d	S
48 Termination	3 2d	3 2d	3 2d	3 2d	2 2d	1 4d	0 3d	0 5d	0 4d	0 4d	0 3d	0 5d
Mean Survival	60%				15%				0%			

Comment codes: d = dead, u = unhealthy

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg NaCl/L)	2240.1
Upper 95% confidence limit (mg NaCl/L)	2378.0
48-hour LC_{50} (mg NaCl/L)	2310.2

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

Acute Daphnid Test-48 Hr Survival

Start Date:	3/4/2025	Test ID:	CdNaClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/6/2025	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	0.6000	0.6000	0.6000
2500	0.4000	0.2000	0.0000	0.0000
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	0.6000	0.6000	0.8861	0.8861	0.8861	0.000	4	10.00	10.00	8	20
*2500	0.1500	0.1500	0.3998	0.2255	0.6847	55.174	4	10.00	10.00	17	20
2750	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4			20	20

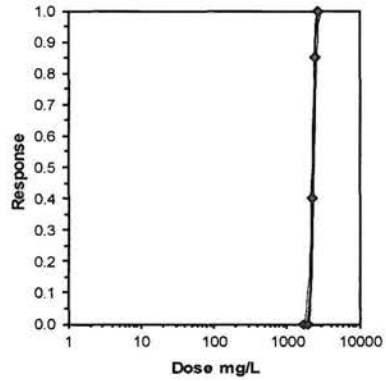
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.59141	0.868	1.10947	6.79713
Equality of variance cannot be confirmed				

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

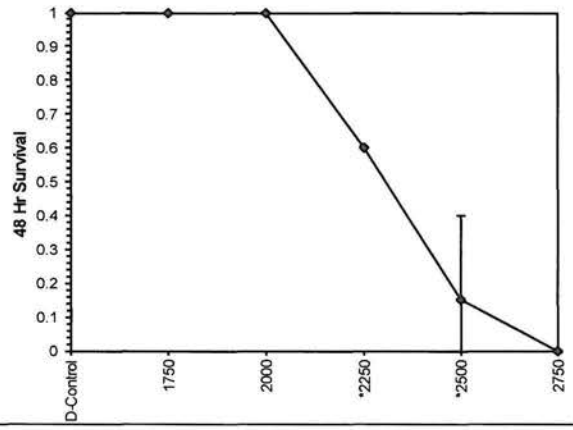
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	34.882	6.76767	21.6173	48.1466	0	0.87708	7.81472	0.83095	3.36364	0.02867	5
Intercept	-112.33	22.7813	-156.98	-67.679							
TSCR											

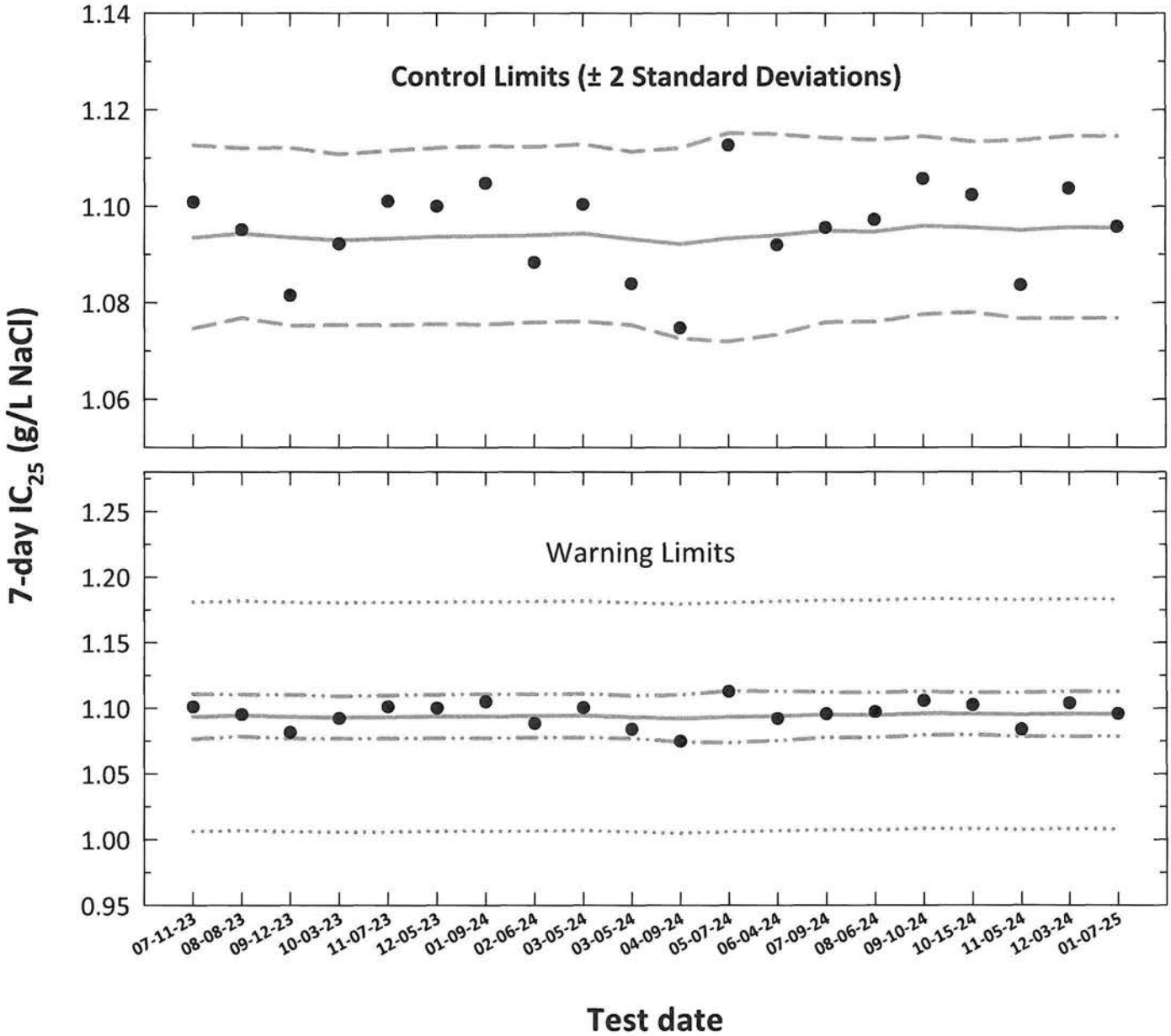
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	1981.31	1786.25	2082.57
EC05	3.355	2072.47	1916.74	2156.05
EC10	3.718	2122.77	1988.87	2197.7
EC15	3.964	2157.4	2038.16	2227.24
EC20	4.158	2185.32	2077.43	2251.83
EC25	4.326	2209.57	2110.99	2273.93
EC40	4.747	2271.85	2193.4	2335.48
EC50	5.000	2310.17	2240.06	2378.02
EC60	5.253	2349.13	2283.57	2425.72
EC75	5.674	2415.35	2348.97	2516.56
EC80	5.842	2442.14	2373.02	2556.15
EC85	6.036	2473.75	2400.12	2604.43
EC90	6.282	2514.1	2433.21	2668.06
EC95	6.645	2575.13	2481.04	2767.54
EC99	7.326	2693.61	2569.41	2968.76



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

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L NaCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L NaCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		10th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.10}$	CT + $S_{A.10}$
1	07-11-23	1.1009	0.0417	0.0388	0.0038	1.0935	1.0747	1.1126	1.0763	1.1107	1.0060	1.1810
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832

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.

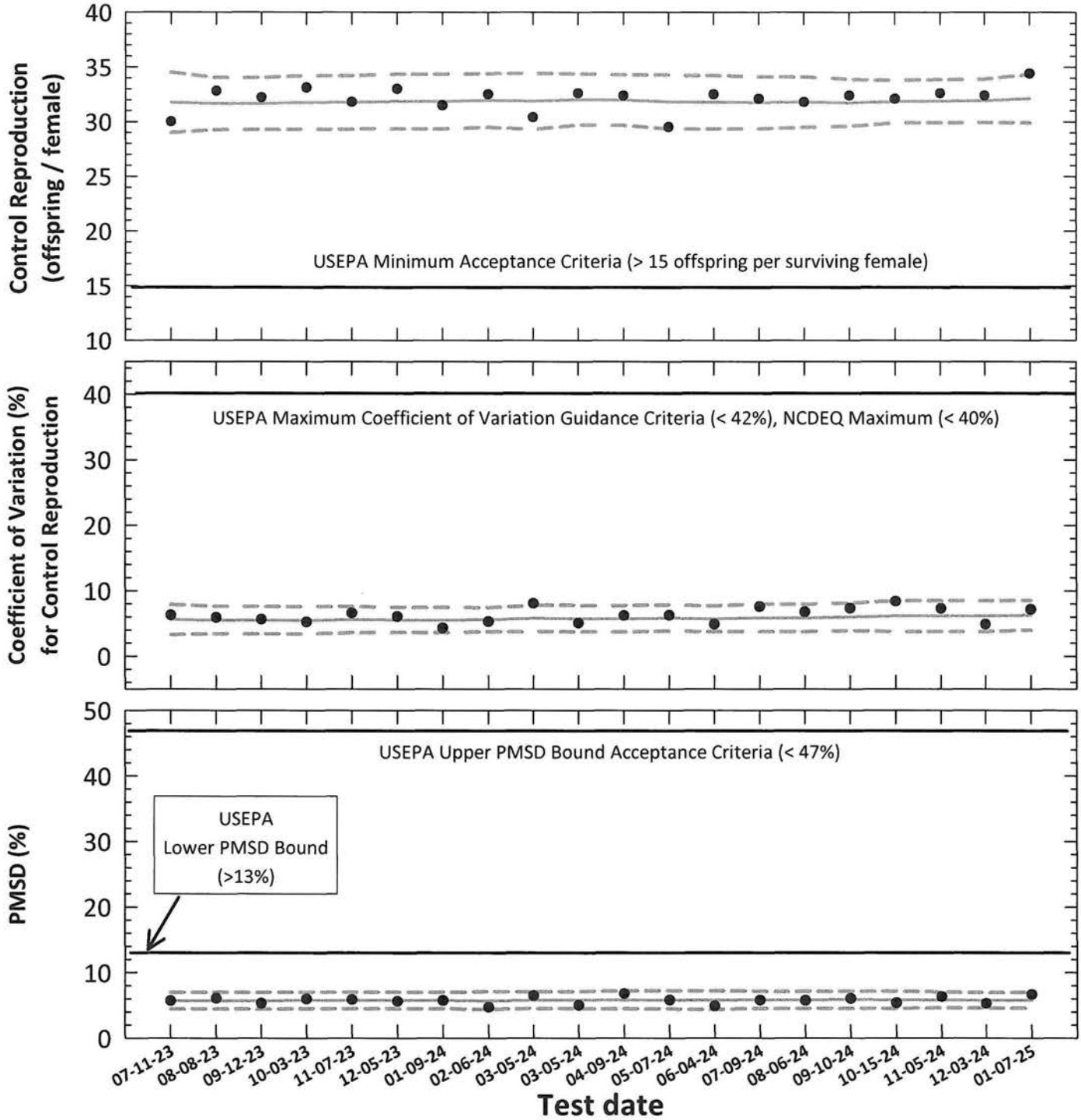
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0

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 #: 306

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2342				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	01.07.25 0515 to 0743									
Culture board:	12-31-24 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	2	3	9	10	11	15	17	18	19	23
Transfer vessel information:	pH (S.U.): 7.95 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	01-07-25	0753	12-26-24	12-26-24	01-02-25B	JH
1	01-08-25	0653	↓	↓	↓	JH
2	01-09-25	0654	↓	↓	01-06-25	JH
3	01-10-25	0654	↓	↓	↓	JH
4	01-11-25	0800	↓	↓	01-07-25	JH
5	01-12-25	0655	↓	↓	↓	JH
6	01-13-25	0723	↓	↓	↓	JH
7	01-14-25	0612				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:	34.4	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	7.17%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1095.8

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 306**

CONTROL ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	6	5	5	6	4	6	6	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	14	16	12	16	14	14	13	11	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	14	17	15	13	19	15	18	14	16
Total young produced		37	34	35	36	32	39	32	35	32	32
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:	34.4

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	5	6	4	6	5	4	4	6	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	15	15	12	15	11	13	13	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	17	18	15	16	15	15	18	16	16	16
Total young produced		35	38	36	32	36	31	35	33	35	34
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	34.5
% Reduction from Control:	-0.3%

Environmental Testing Solutions, Inc.

Species: *Ceriodaphnia dubia* **CdNaClCR #: 306**

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	6	5	4	4	5	5	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	15	11	15	14	14	12	14	13	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	16	16	19	17	15	16	15	19	15	19
Total young produced		36	30	40	36	33	32	34	37	33	36
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	34.7
% Reduction from Control:	-0.9%

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	6	5	5	4	4	4	4	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	13	10	13	10	12	14	14	13	14
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	13	16	14	17	15	19	17	17	13	15
Total young produced		31	34	29	34	29	35	35	35	31	34
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	32.7
% Reduction from Control:	4.9%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 306**

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	4	4	3	5	4	4	4	6	3
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	11	9	7	10	11	8	5	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	5	6	3	9	5	4	3	10	2	4
Total young produced		19	21	16	19	20	19	15	19	20	17
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	18.5
% Reduction from Control:	46.2%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2.1
% Reduction from Control:	93.9%

Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	6	5	5	6	4	6	6	4	51
5	14	16	12	16	14	14	13	11	12	12	134
6	0	0	0	0	0	0	0	0	0	0	0
7	18	14	17	15	13	19	15	18	14	16	159
Total	**37**	**34**	**35**	**36**	**32**	**39**	**32**	**35**	**32**	**32**	**344**

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	5	5	4	4	4	4	4	5	5	46
5	12	13	10	13	10	12	14	14	13	14	125
6	0	0	0	0	0	0	0	0	0	0	0
7	13	16	14	17	15	19	17	17	13	15	156
Total	**31**	**34**	**29**	**34**	**29**	**35**	**35**	**35**	**31**	**34**	**327**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	5	6	4	6	5	4	4	6	6	51
5	13	15	15	12	15	11	13	13	13	12	132
6	0	0	0	0	0	0	0	0	0	0	0
7	17	18	15	16	15	15	18	16	16	16	162
Total	**35**	**38**	**36**	**32**	**36**	**31**	**35**	**33**	**35**	**34**	**345**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	3	5	4	4	4	6	3	42
5	9	11	9	7	10	11	8	5	12	10	92
6	0	0	0	0	0	0	0	0	0	0	0
7	5	6	3	9	5	4	3	10	2	4	51
Total	**19**	**21**	**16**	**19**	**20**	**19**	**15**	**19**	**20**	**17**	**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	3	6	5	4	4	5	5	5	5	47
5	15	11	15	14	14	12	14	13	13	12	133
6	0	0	0	0	0	0	0	0	0	0	0
7	16	16	19	17	15	16	15	19	15	19	167
Total	**36**	**30**	**40**	**36**	**33**	**32**	**34**	**37**	**33**	**36**	**347**

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	1	1	2	1	3	3	1	1	18
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	1	0	0	0	0	2	0	0	3
Total	**3**	**2**	**2**	**1**	**2**	**1**	**3**	**5**	**1**	**1**	**21**

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: 306

Test dates: January 07-14, 2025

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	37	34	35	36	32	39	32	35	32	32	100	34.4	7.1	Not applicable
600	35	38	36	32	36	31	35	33	35	34	100	34.5	6.0	-0.3
800	36	30	40	36	33	32	34	37	33	36	100	34.7	8.3	-0.9
1000	31	34	29	34	29	35	35	35	31	34	100	32.7	7.5	4.9
1200	19	21	16	19	20	19	15	19	20	17	100	18.5	10.3	46.2
1400	3	2	2	1	2	1	3	5	1	1	100	2.1	61.3	93.9

Dunnett's MSD value: 2.280

PMSD: 6.6

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

Lower PMSD bound determined by USEPA (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:	1/7/2025	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	1/14/2025	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	37.000	34.000	35.000	36.000	32.000	39.000	32.000	35.000	32.000	32.000
600	35.000	38.000	36.000	32.000	36.000	31.000	35.000	33.000	35.000	34.000
800	36.000	30.000	40.000	36.000	33.000	32.000	34.000	37.000	33.000	36.000
1000	31.000	34.000	29.000	34.000	29.000	35.000	35.000	35.000	31.000	34.000
1200	19.000	21.000	16.000	19.000	20.000	19.000	15.000	19.000	20.000	17.000
1400	3.000	2.000	2.000	1.000	2.000	1.000	3.000	5.000	1.000	1.000

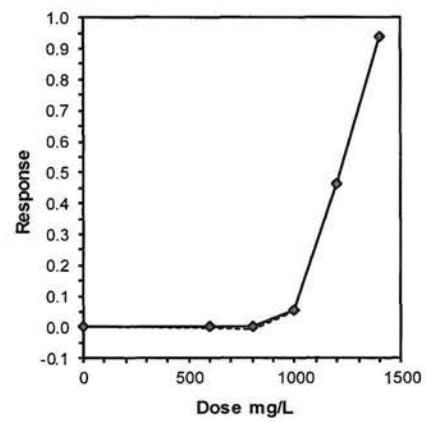
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	34.400	1.0000	34.400	32.000	39.000	7.147	10				34.533	1.0000
600	34.500	1.0029	34.500	31.000	38.000	5.995	10	-0.100	2.287	2.280	34.533	1.0000
800	34.700	1.0087	34.700	30.000	40.000	8.269	10	-0.301	2.287	2.280	34.533	1.0000
1000	32.700	0.9506	32.700	29.000	35.000	7.498	10	1.705	2.287	2.280	32.700	0.9469
*1200	18.500	0.5378	18.500	15.000	21.000	10.272	10	15.944	2.287	2.280	18.500	0.5357
*1400	2.100	0.0610	2.100	1.000	5.000	61.271	10	32.390	2.287	2.280	2.100	0.0608

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.98847	1.035	-0.0009	-0.2805
Bartlett's Test indicates equal variances (p = 0.32)	5.87233	15.0863		

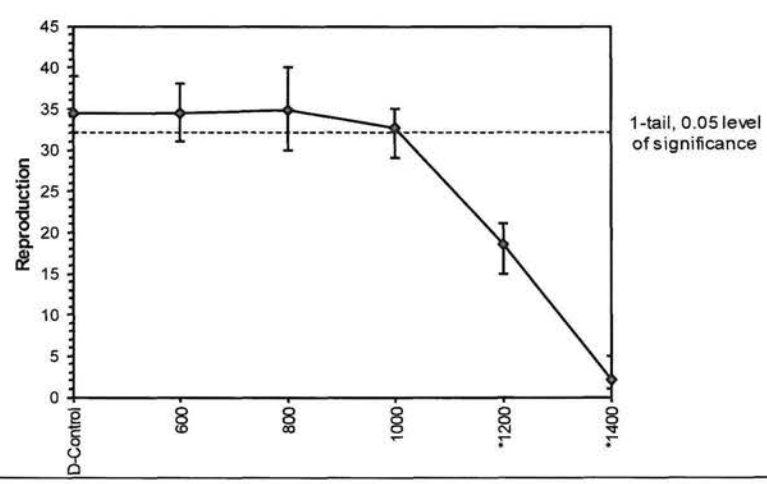
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		2.28031	0.06629	1781.43	4.97222	4.2E-40	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	988.364	69.1745	782.089	1020.42	-1.2880
IC10	1022.82	16.2455	973.21	1043.03	-1.1306
IC15	1047.14	11.6651	1019.62	1065.64	-0.0580
IC20	1071.46	10.5698	1047.8	1088.26	-0.0236
IC25	1095.77	9.63103	1074.62	1110.87	0.0018
IC40	1168.73	8.25902	1150.27	1180.96	-0.0783
IC50	1215.04	7.03833	1198.23	1224.49	-0.4623



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 306**

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.

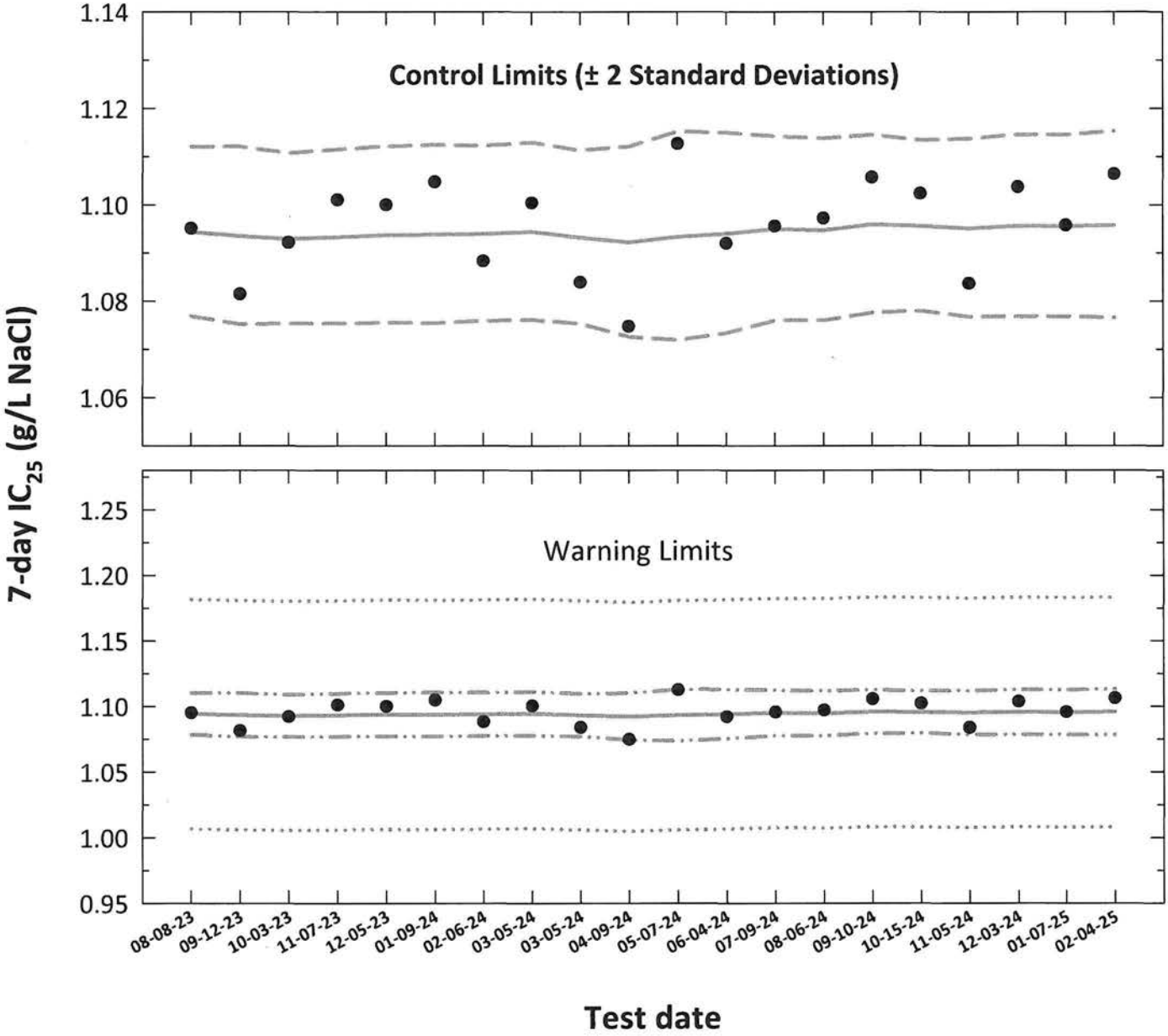
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	EM	EM	EM	EM	EM	EM
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.00	8.02	8.02	8.01	8.01	8.00
	Dissolved oxygen (mg/L)	7.6	7.6	7.8	8.0	8.0	7.9
	Conductivity (µmhos/cm)	316		308		312	
	Alkalinity (mg $CaCO_3$/L)	60		0-6-25		58	
	Hardness (mg $CaCO_3$/L)	87				89	
	Temperature (°C)	25.0	25.2	24.8	25.2	24.8	25.1
600 mg NaCl/L	pH (S.U.)	8.03	7.98	8.02	7.98	8.04	7.98
	Dissolved oxygen (mg/L)	7.6	7.6	7.8	8.0	8.0	7.9
	Conductivity (µmhos/cm)	1470		1450		1470	
	Temperature (°C)	25.1	25.0	24.9	25.0	24.9	24.8
800 mg NaCl/L	pH (S.U.)	8.04	7.98	8.01	7.99	8.03	7.99
	Dissolved oxygen (mg/L)	7.7	7.5	7.8	8.0	8.0	7.9
	Conductivity (µmhos/cm)	1870		1840		1860	
	Temperature (°C)	24.9	25.0	24.9	25.0	24.9	24.8
1000 mg NaCl/L	pH (S.U.)	8.04	7.99	8.01	7.98	8.03	7.99
	Dissolved oxygen (mg/L)	7.7	7.5	7.8	8.0	8.0	7.9
	Conductivity (µmhos/cm)	2260		2210		2250	
	Temperature (°C)	24.9	24.9	24.9	24.9	24.8	25.0
1200 mg NaCl/L	pH (S.U.)	8.05	7.98	8.02	7.98	8.03	7.99
	Dissolved oxygen (mg/L)	7.8	7.5	7.8	8.0	8.0	7.9
	Conductivity (µmhos/cm)	2650		2580		2630	
	Temperature (°C)	24.9	24.9	25.0	24.9	24.8	25.0
1400 mg NaCl/L	pH (S.U.)	8.05	7.99	8.02	7.97	8.03	7.99
	Dissolved oxygen (mg/L)	7.8	7.5	7.7	8.0	8.1	7.8
	Conductivity (µmhos/cm)	3000		2920		2980	
	Temperature (°C)	24.9	25.1	25.0	24.9	24.8	24.8
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 306**

		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.)	8.03	8.05	8.10	8.08	8.21	7.95	7.93	7.94
	Dissolved oxygen (mg/L)	7.7	7.9	7.7	7.7	8.0	7.9	8.0	7.7
	Conductivity (µmhos/cm)	305		291		307		312	
	Alkalinity (mg $CaCO_3$/L)	Kaus-15		59				Kaus-15	
	Hardness (mg $CaCO_3$/L)			81					
	Temperature (°C)	24.9	25.2	24.8	25.1	24.7	25.0	24.9	25.2
600 mg NaCl/L	pH (S.U.)	8.04	8.03	8.10	8.04	8.11	7.95	7.99	7.98
	Dissolved oxygen (mg/L)	7.7	7.9	7.8	7.7	8.0	7.9	8.0	7.7
	Conductivity (µmhos/cm)	1450		1580		1590		1680	
	Temperature (°C)	25.0	25.0	24.9	25.1	24.7	24.8	24.9	25.2
800 mg NaCl/L	pH (S.U.)	8.04	8.04	8.10	8.05	8.12	7.94	8.01	7.97
	Dissolved oxygen (mg/L)	7.7	7.9	7.8	7.6	8.0	7.9	8.0	7.9
	Conductivity (µmhos/cm)	1860		1730		1780		1880	
	Temperature (°C)	25.0	25.0	24.9	25.0	24.7	24.8	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	8.03	8.05	8.10	8.04	8.11	7.95	8.01	7.98
	Dissolved oxygen (mg/L)	7.7	7.9	7.8	7.6	8.0	7.9	7.9	7.9
	Conductivity (µmhos/cm)	2250		2090		2140		2260	
	Temperature (°C)	24.9	25.0	25.0	25.3	24.8	24.8	25.0	25.0
1200 mg NaCl/L	pH (S.U.)	8.04	8.05	8.09	8.03	8.10	7.95	8.01	7.98
	Dissolved oxygen (mg/L)	7.7	8.0	7.9	7.7	8.0	7.9	8.0	7.9
	Conductivity (µmhos/cm)	2620		2450		2540		2650	
	Temperature (°C)	24.9	24.9	25.0	25.2	25.0	25.1	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	8.04	8.05	8.08	8.04	8.11	7.95	8.01	7.99
	Dissolved oxygen (mg/L)	7.6	8.0	7.9	7.7	8.0	8.0	8.0	7.9
	Conductivity (µmhos/cm)	2980		2810		2890		3030	
	Temperature (°C)	24.9	24.9	25.0	25.2	25.0	24.9	24.9	25.1
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



- ● **7-day IC_{25}** = 25% inhibition concentration. An estimation of the sodium chloride concentration which would cause a 25% reduction in *Ceriodaphnia* reproduction (calculated using ToxCalc).
- **Central Tendency** (mean logarithmic IC_{25} converted to anti-logarithmic values)
- **Control Limits** (mean logarithmic IC_{25} ± 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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
20	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835

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

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

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

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

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

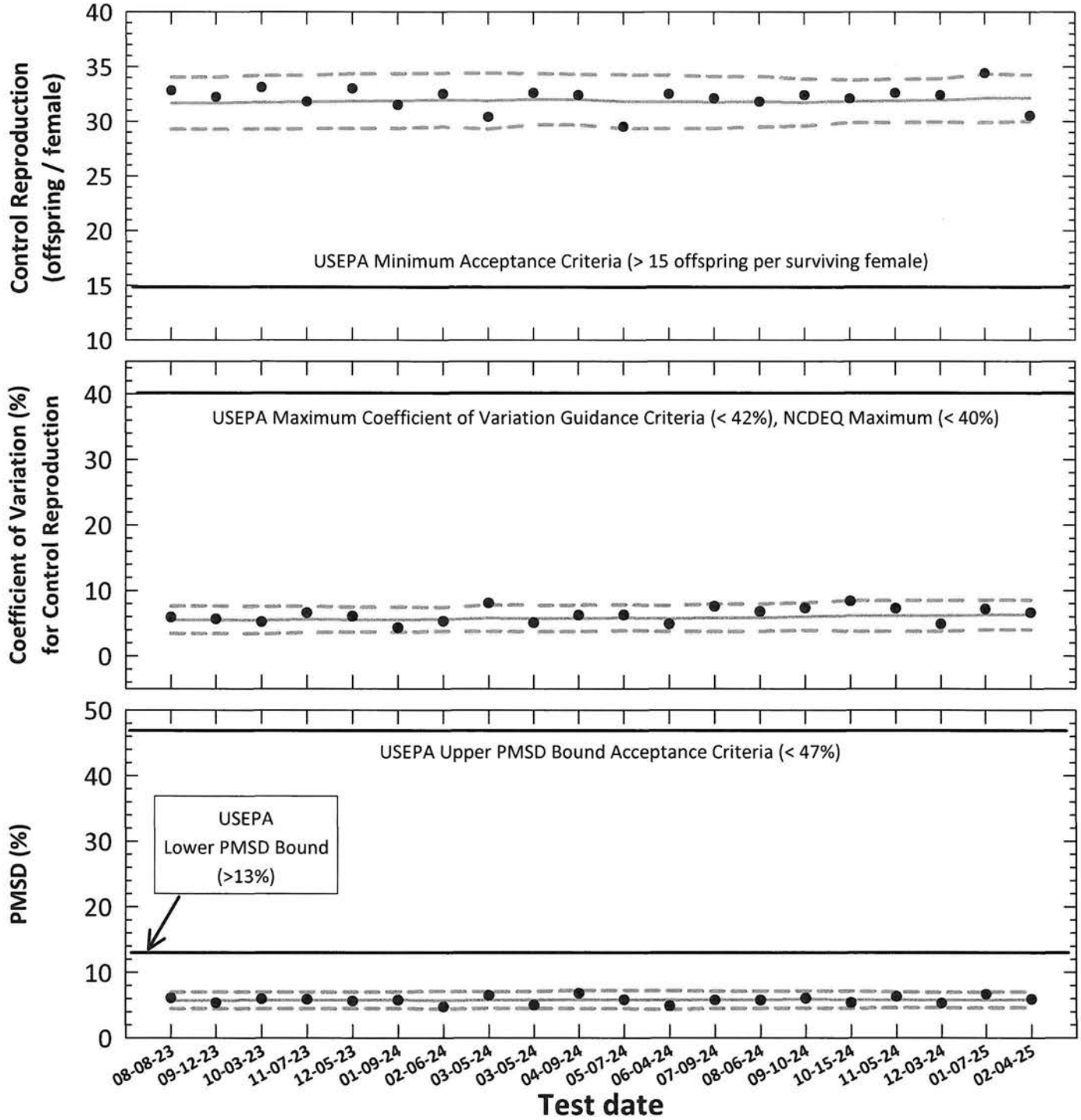
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

CV = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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



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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

Test number	Test date	ToxCal Determination					Control Reproduction (offspring/female)			Control Reproduction CV (%)			Test PMSD (%)		
		Control	Control Reproduction		Test										
		Survival (%)	Mean (offspring/female)	CV (%)	MSD	PMSD (%)	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
20	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0

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 #: 307

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2342				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	02-04-25 0415 TO 0820									
Culture board:	01-28-25 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	2	4	5	6	7	8	10	11	14	15
Transfer vessel information:	pH (S.U.): 8.17 Temperature (°C): 24.9									
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	02-04-25	0832	01-30-25	01-30-25	01-28-25 B	JH
1	02-05-25	0625	↓	↓	↓	JH
2	02-06-25	0626	↓	↓	02-02-25 B	JH
3	02-07-25	0630	↓	↓	↓	JH
4	02-08-25	0100	↓	↓	02-06-25 A	JH
5	02-09-25	0600	↓	↓	↓	JH
6	02-10-25	0626	↓	↓	↓	JH
7	02-11-25	0733				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	130664665

Control information:		Acceptance criteria	***Summary of test endpoints:***	
% of Male Adults:	0%	≤ 20%	**7-day LC_{50}** (mg/L NaCl)	>1400
% Adults having 3rd Broods:	100%	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	30.5	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	6.6%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1106.4

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 307**

CONTROL ***Survival and Reproduction Data***

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	3	4	5	5	5	4	4	4	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	11	11	13	9	10	12	12	10	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	17	13	13	14	16	15	18	16	17	14
Total young produced		30	27	28	32	30	30	34	32	31	31
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.5

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.1
% Reduction from Control:	-2.0%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 307**

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	3	4	4	6	6	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	13	10	10	10	12	11	13	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	16	17	17	13	18	15	14	16	13
Total young produced		32	30	31	30	29	33	34	30	32	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:	31.0
% Reduction from Control:	-1.6%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	29.8
% Reduction from Control:	2.3%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 307**

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	5	4	4	4	3	3	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	6	6	9	5	10	5	8	4	7	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	5	8	7	6	6	10	5	11	3	9
Total young produced		14	18	19	16	20	19	17	18	13	19
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	17.3
% Reduction from Control:	45.3%

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	1.9
% Reduction from Control:	93.8%

Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	4	5	5	5	4	4	4	5	43
5	9	11	11	13	9	10	12	12	10	12	109
6	0	0	0	0	0	0	0	0	0	0	0
7	17	13	13	14	16	15	18	16	17	14	153
Total	**30**	**27**	**28**	**32**	**30**	**30**	**34**	**32**	**31**	**31**	**305**

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	3	3	5	4	4	4	4	4	41
5	11	12	12	9	12	10	11	12	10	10	109
6	0	0	0	0	0	0	0	0	0	0	0
7	14	12	14	16	13	17	16	16	15	15	148
Total	**31**	**28**	**29**	**28**	**30**	**31**	**31**	**32**	**29**	**29**	**298**

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	3	4	5	4	5	6	5	4	4	45
5	9	12	11	12	10	12	13	12	12	10	113
6	0	0	0	0	0	0	0	0	0	0	0
7	16	17	13	15	16	14	13	18	15	16	153
Total	**30**	**32**	**28**	**32**	**30**	**31**	**32**	**35**	**31**	**30**	**311**

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	5	4	4	4	3	3	4	37
5	6	6	9	5	10	5	8	4	7	6	66
6	0	0	0	0	0	0	0	0	0	0	0
7	5	8	7	6	6	10	5	11	3	9	70
Total	**14**	**18**	**19**	**16**	**20**	**19**	**17**	**18**	**13**	**19**	**173**

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	3	4	4	6	6	4	4	44
5	13	10	10	10	12	11	13	10	12	12	113
6	0	0	0	0	0	0	0	0	0	0	0
7	14	16	17	17	13	18	15	14	16	13	153
Total	**32**	**30**	**31**	**30**	**29**	**33**	**34**	**30**	**32**	**29**	**310**

1400 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	3	1	1	1	1	3	2	2	17
5	0	0	0	1	0	0	1	0	0	0	2
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
Total	**2**	**1**	**3**	**2**	**1**	**1**	**2**	**3**	**2**	**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: 307

Test dates: February 04-11, 2025

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	30	27	28	32	30	30	34	32	31	31	**100**	**30.5**	**6.6**	**Not applicable**
600	30	32	28	32	30	31	32	35	31	30	**100**	**31.1**	**6.0**	**-2.0**
800	32	30	31	30	29	33	34	30	32	29	**100**	**31.0**	**5.5**	**-1.6**
1000	31	28	29	28	30	31	31	32	29	29	**100**	**29.8**	**4.7**	**2.3**
1200	14	18	19	16	20	19	17	18	13	19	**100**	**17.3**	**13.4**	**43.3**
1400	2	1	3	2	1	1	2	3	2	2	**100**	**1.9**	**38.8**	**93.8**

Dunnett's MSD value: 1.782

PMSD: 5.8

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

Lower PMSD bound determined by USEPA (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 Sumner

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	2/4/2025	Test ID:	CdNaCICR	Sample ID:	REF-Ref Toxicant
End Date:	2/11/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
D-Control	30.000	27.000	28.000	32.000	30.000	30.000	34.000	32.000	31.000	31.000
600	30.000	32.000	28.000	32.000	30.000	31.000	32.000	35.000	31.000	30.000
800	32.000	30.000	31.000	30.000	29.000	33.000	34.000	30.000	32.000	29.000
1000	31.000	28.000	29.000	28.000	30.000	31.000	31.000	32.000	29.000	29.000
1200	14.000	18.000	19.000	16.000	20.000	19.000	17.000	18.000	13.000	19.000
1400	2.000	1.000	3.000	2.000	1.000	1.000	2.000	3.000	2.000	2.000

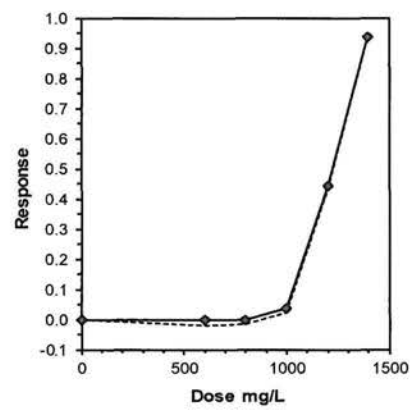
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	30.500	1.0000	30.500	27.000	34.000	6.603	10				30.867	1.0000
600	31.100	1.0197	31.100	28.000	35.000	5.958	10	-0.770	2.287	1.782	30.867	1.0000
800	31.000	1.0164	31.000	29.000	34.000	5.483	10	-0.642	2.287	1.782	30.867	1.0000
1000	29.800	0.9770	29.800	28.000	32.000	4.693	10	0.898	2.287	1.782	29.800	0.9654
*1200	17.300	0.5672	17.300	13.000	20.000	13.363	10	16.937	2.287	1.782	17.300	0.5605
*1400	1.900	0.0623	1.900	1.000	3.000	38.835	10	36.697	2.287	1.782	1.900	0.0616

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.69067	1.035	-0.1616	0.27141
Bartlett's Test indicates equal variances (p = 0.06)	10.6293	15.0863		

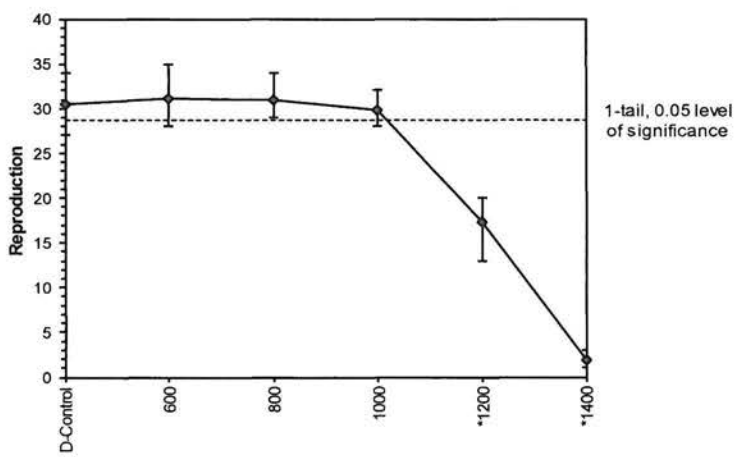
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.78214	0.05843	1415.28	3.03704	4.2E-43	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	1007.63	47.3037	927.563	1020.37	-8.8139
IC10	1032.32	7.27028	1017.73	1044.87	-0.1136
IC15	1057.01	6.87923	1043.13	1068.16	-0.1332
IC20	1081.71	6.84561	1067.52	1092.7	-0.1490
IC25	1106.4	7.17445	1091.02	1118.31	-0.1517
IC40	1180.48	9.76716	1161.12	1198.23	-0.1100
IC50	1224.24	7.9653	1207.98	1236.44	-0.6021



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 307**

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	M
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.05	7.87	7.82	7.93	7.91	7.98
	Dissolved oxygen (mg/L)	8.0	7.8	7.9	7.8	7.9	7.8
	Conductivity (µmhos/cm)	300		312		306	
	Alkalinity (mg $CaCO_3$/L)	59		01-12-25		60	
	Hardness (mg $CaCO_3$/L)	90				86	
	Temperature (°C)	24.8	25.2	24.9	25.1	24.8	25.1
600 mg NaCl/L	pH (S.U.)	8.01	7.84	7.87	7.88	7.94	7.89
	Dissolved oxygen (mg/L)	7.9	7.8	7.9	7.8	7.9	7.9
	Conductivity (µmhos/cm)	1430		1460		1450	
	Temperature (°C)	24.9	25.0	24.9	24.9	24.9	25.2
800 mg NaCl/L	pH (S.U.)	8.01	7.83	7.88	7.88	7.94	7.89
	Dissolved oxygen (mg/L)	7.9	7.7	7.8	7.8	7.9	7.9
	Conductivity (µmhos/cm)	1780		1830		1840	
	Temperature (°C)	24.9	25.0	25.0	24.9	24.9	25.2
1000 mg NaCl/L	pH (S.U.)	8.00	7.83	7.86	7.89	7.92	7.89
	Dissolved oxygen (mg/L)	7.9	7.7	7.9	7.8	8.0	8.0
	Conductivity (µmhos/cm)	2140		2180		2180	
	Temperature (°C)	25.0	25.0	25.0	24.9	24.9	25.0
1200 mg NaCl/L	pH (S.U.)	7.99	7.83	7.87	7.88	7.93	7.89
	Dissolved oxygen (mg/L)	8.0	7.7	7.8	7.8	7.9	8.0
	Conductivity (µmhos/cm)	2510		2550		2540	
	Temperature (°C)	24.9	25.1	24.9	25.0	25.0	25.0
1400 mg NaCl/L	pH (S.U.)	7.99	7.82	7.88	7.88	7.93	7.88
	Dissolved oxygen (mg/L)	8.0	7.7	7.8	7.9	8.0	8.0
	Conductivity (µmhos/cm)	2900		2870		2870	
	Temperature (°C)	24.9	25.2	24.9	25.0	24.9	25.0
		Initial	Final	Initial	Final	Initial	Final

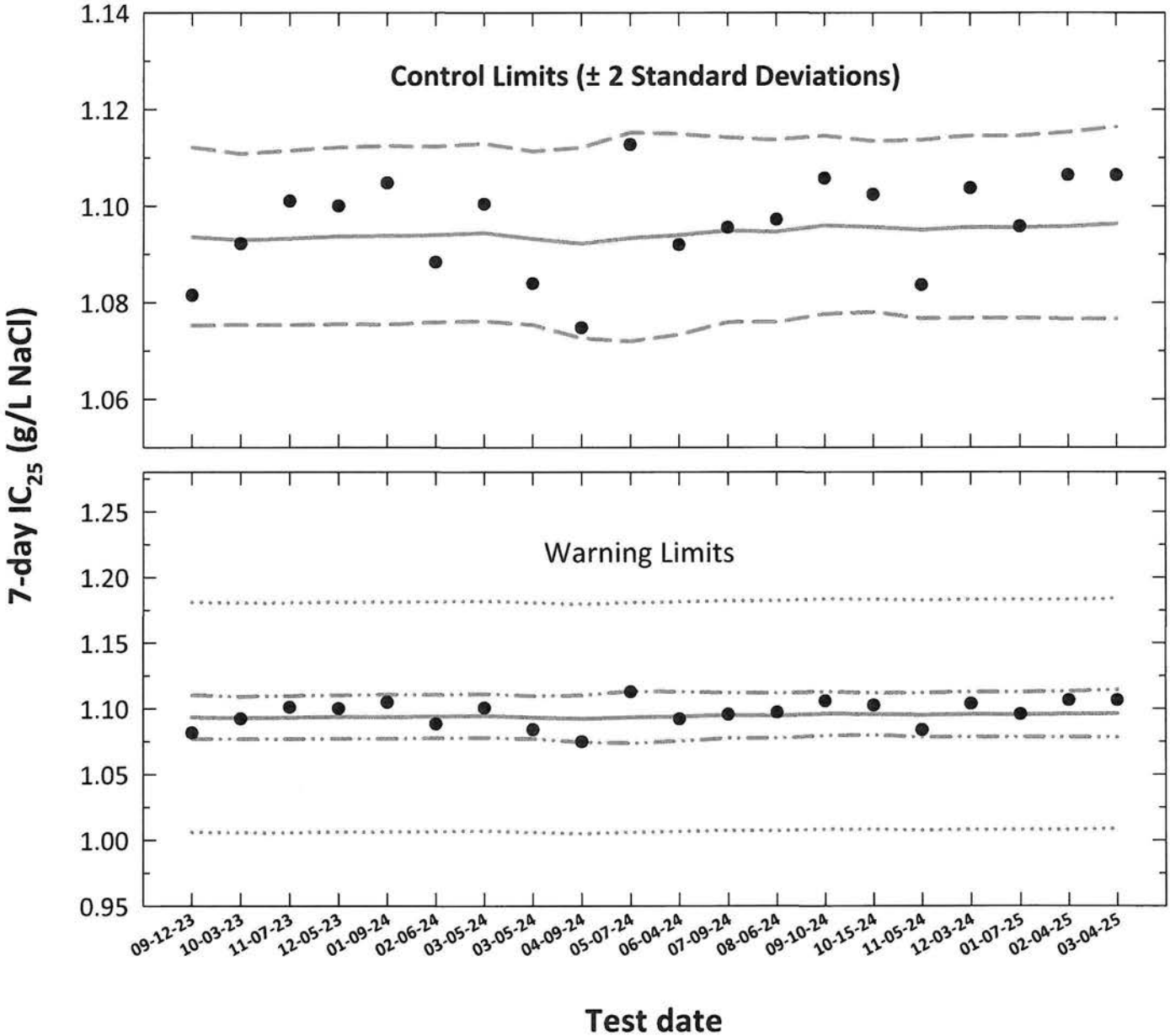
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia***

CdNaClCR #: 307

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	N	N	N	N	N	EM	EM	N
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	8.02	8.02	8.10	7.98	8.09	7.98	7.97	8.12
	Dissolved oxygen (mg/L)	7.8	7.6	7.9	7.7	8.0	7.9	8.1	7.6
	Conductivity (µmhos/cm)	300		293		296		310	
	Alkalinity (mg $CaCO_3$/L)			59					
	Hardness (mg $CaCO_3$/L)			88					
	Temperature (°C)	24.7	25.2	24.8	24.9	24.8	25.1	24.8	25.2
600 mg NaCl/L	pH (S.U.)	7.98	7.93	8.00	7.91	7.94	8.00	8.02	8.02
	Dissolved oxygen (mg/L)	7.9	7.6	7.9	7.7	8.0	7.9	8.1	7.6
	Conductivity (µmhos/cm)	1400		1380		1360		1470	
	Temperature (°C)	24.8	25.2	24.9	25.1	24.9	24.9	24.9	24.9
800 mg NaCl/L	pH (S.U.)	7.99	7.92	7.99	7.90	7.93	8.01	8.04	8.01
	Dissolved oxygen (mg/L)	7.9	7.6	8.0	7.7	8.0	7.9	8.1	7.6
	Conductivity (µmhos/cm)	1760		1740		1740		1860	
	Temperature (°C)	24.9	25.0	24.9	25.1	24.9	24.9	24.9	24.9
1000 mg NaCl/L	pH (S.U.)	7.98	7.93	8.01	7.90	7.93	8.01	8.03	8.01
	Dissolved oxygen (mg/L)	7.9	7.6	8.0	7.7	8.0	7.9	8.0	7.6
	Conductivity (µmhos/cm)	2100		2080		2080		2210	
	Temperature (°C)	24.9	24.9	24.9	24.8	25.0	25.2	25.0	24.9
1200 mg NaCl/L	pH (S.U.)	7.97	7.91	8.00	7.90	7.92	8.01	8.04	8.00
	Dissolved oxygen (mg/L)	7.9	7.6	8.1	7.7	8.1	8.0	8.0	7.6
	Conductivity (µmhos/cm)	2450		2440		2420		2600	
	Temperature (°C)	24.9	24.9	25.0	24.9	25.0	25.0	24.9	24.9
1400 mg NaCl/L	pH (S.U.)	7.99	7.90	8.01	7.90	7.93	8.01	8.04	8.00
	Dissolved oxygen (mg/L)	7.9	7.6	8.1	7.6	8.1	8.0	8.1	7.6
	Conductivity (µmhos/cm)	2770		2760		2750		2910	
	Temperature (°C)	24.9	24.9	25.0	25.2	25.0	25.2	24.9	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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
19	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
20	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841

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.

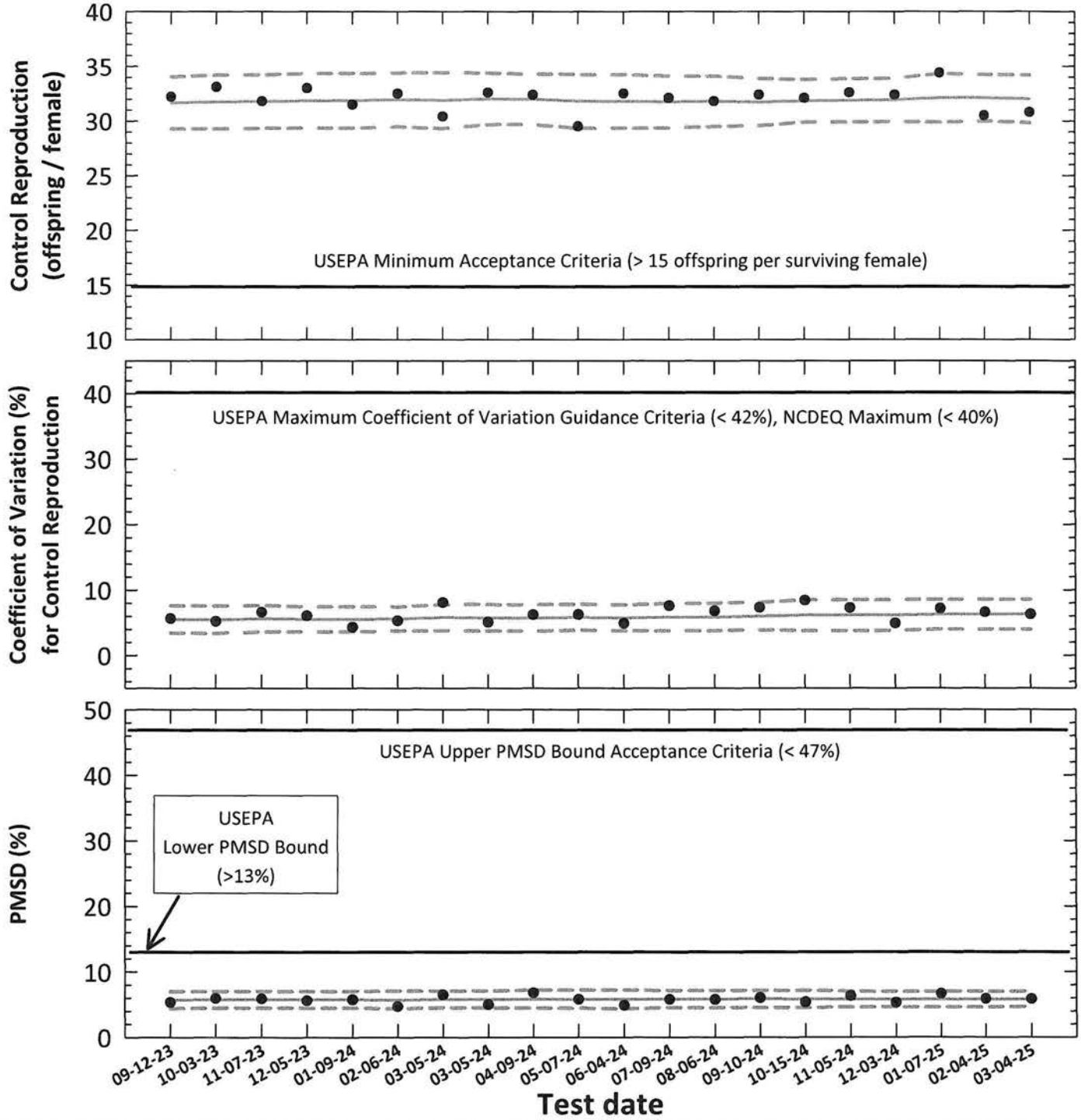
$S_{A.10}$ = Standard deviation corresponding to the 10th percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

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	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S	CT	95% Confidence Interval CT - 2S	CT + 2S
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
19	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
20	03-04-25	100	30.8	6.3	1.797	5.8	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0

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 #: 308

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2356				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	03·04·25 0530 TO 0800									
Culture board:	02-25-25 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	4	6	8	12	13	14	15	17	18	19
Transfer vessel information:	pH (S.U.): 7.91 Temperature (°C): 25.1									
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	03-04-25	0810	02-21-25	01-30-25	02-26-25 A	H
1	03-05-25	0610	↓	↓	↓	H
2	03-06-25	0622	↓	↓	03-03-26 A	H
3	03-07-25	0610	↓	↓	↓	H
4	03-08-25	0622	↓	↓	03-06-25 A	H
5	03-09-25	0610	↓	↓	↓	H
6	03-10-25	0610	↓	↓	↓	H
7	03-11-25	0610				H

*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% ~~30.8~~	≥ 80%	**NOEC** (mg/L NaCl)	1000
% Mortality:	0%	≤ 20%	**LOEC** (mg/L NaCl)	1200
Mean Offspring/Female:	~~6.3~~ 30.8	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	6.3%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1106.4

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 308**

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	6	4	4	4	5	5	6	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	11	10	11	12	10	10	10	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	16	13	16	15	15	14	18	17	15	18
Total young produced		33	28	30	30	32	29	34	31	29	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L
X for 3rd Broods		X	X	X	X	X	X	X	X	X	X

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.8

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	5	6	4	4	5	5	5	5	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	10	11	12	10	10	10	10	12	12	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	15	19	17	17	14	15	17	14	19
Total young produced		30	32	35	31	32	29	30	34	30	33
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	31.6
% Reduction from Control:	-2.6%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 308**

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	6	6	5	4	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	11	11	11	13	10	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	14	16	17	16	18	15	15	15	18	14
Total young produced		31	30	34	33	34	32	29	31	32	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:	~~31.6~~ 31.5
% Reduction from Control:	-2.3%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	29.7
% Reduction from Control:	3.6%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 308**

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	3	4	4	5	4	4	4	4	4
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	9	6	5	8	10	4	7	10	6	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	11	8	5	5	10	7	3	9	5
Total young produced		18	20	17	17	20	18	18	17	19	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:	18.0
% Reduction from Control:	41.6%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	1.7
% Reduction from Control:	94.5%

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	6	4	4	4	5	5	6	4	4	4	46
5	11	11	10	11	12	10	10	10	10	10	105
6	0	0	0	0	0	0	0	0	0	0	0
7	16	13	16	15	15	14	18	17	15	18	157
Total	**33**	**28**	**30**	**30**	**32**	**29**	**34**	**31**	**29**	**32**	**308**

1000 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	3	6	4	4	5	5	4	5	4	45
5	10	9	12	10	10	10	11	11	11	10	104
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	13	16	13	15	17	14	17	13	148
Total	**30**	**27**	**31**	**30**	**27**	**30**	**33**	**29**	**33**	**27**	**297**

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	5	5	5	5	4	4	47
5	10	11	12	10	10	10	10	12	12	10	107
6	0	0	0	0	0	0	0	0	0	0	0
7	15	15	19	17	17	14	15	17	14	19	162
Total	**30**	**32**	**35**	**31**	**32**	**29**	**30**	**34**	**30**	**33**	**316**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	3	4	4	5	4	4	4	4	4	40
5	9	6	5	8	10	4	7	10	6	7	72
6	0	0	0	0	0	0	0	0	0	0	0
7	5	11	8	5	5	10	7	3	9	5	68
Total	**18**	**20**	**17**	**17**	**20**	**18**	**18**	**17**	**19**	**16**	**180**

800 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	5	4	6	6	5	4	4	4	4	4	46
5	12	10	11	11	11	13	10	12	10	11	111
6	0	0	0	0	0	0	0	0	0	0	0
7	14	16	17	16	18	15	15	15	18	14	158
Total	**31**	**30**	**34**	**33**	**34**	**32**	**29**	**31**	**32**	**29**	**315**

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	3	0	1	1	1	2	1	16
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**	**3**	**1**	**1**	**1**	**1**	**2**	**1**	**17**

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

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

Test number: 308

Test dates: March 04-11, 2025

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	33	28	30	30	32	29	34	31	29	32	**100**	**30.8**	**6.3**	**Not applicable**
600	30	32	35	31	32	29	30	34	30	33	**100**	**31.6**	**6.2**	**-2.6**
800	31	30	34	33	34	32	29	31	32	29	**100**	**31.5**	**5.8**	**-2.3**
1000	30	27	31	30	27	30	33	29	33	27	**100**	**29.7**	**7.6**	**3.6**
1200	18	20	17	17	20	18	18	17	19	16	**100**	**18.0**	**7.4**	**41.6**
1400	3	2	2	3	1	1	1	1	2	1	**100**	**1.7**	**48.4**	**94.5**

Dunnett's MSD value: 1.797

PMSD: 5.8

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

Lower PMSD bound determined by USEPA (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:	3/4/2025	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	3/11/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

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

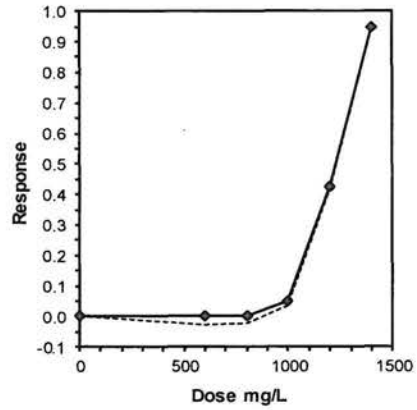
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	30.800	1.0000	30.800	28.000	34.000	6.273	10				31.300	1.0000
600	31.600	1.0260	31.600	29.000	35.000	6.187	10	-1.018	2.287	1.797	31.300	1.0000
800	31.500	1.0227	31.500	29.000	34.000	5.844	10	-0.891	2.287	1.797	31.300	1.0000
1000	29.700	0.9643	29.700	27.000	33.000	7.620	10	1.400	2.287	1.797	29.700	0.9489
*1200	18.000	0.5844	18.000	16.000	20.000	7.407	10	16.290	2.287	1.797	18.000	0.5751
*1400	1.700	0.0552	1.700	1.000	3.000	48.428	10	37.035	2.287	1.797	1.700	0.0543

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.65597	1.035	0.22771	-0.6268
Bartlett's Test indicates equal variances (p = 0.10)	9.18469	15.0863		

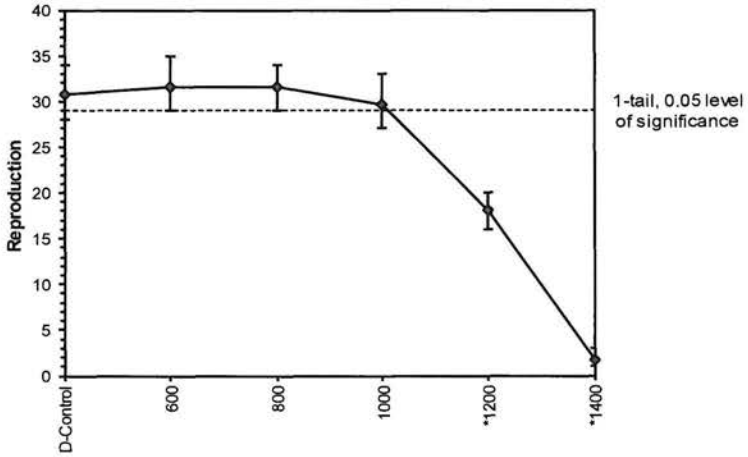
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1000	1200	1095.45		1.79675	0.05834	1451.9	3.08704	3.3E-43	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	995.625	42.6753	888.134	1022.63	-1.3798
IC10	1026.15	11.3195	1004.52	1047.01	-0.3324
IC15	1052.91	9.73378	1033.15	1072.12	-0.0203
IC20	1079.66	8.61056	1062.37	1096.83	0.0359
IC25	1106.41	7.71411	1090.97	1121.88	0.0805
IC40	1186.67	7.05511	1171.44	1200.15	0.0308
IC50	1228.83	4.63868	1218.58	1237.14	-0.2300



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 308**

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	Em	Em	Em	Em	Em	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.96	7.86	7.86	7.98	7.93	7.99
	Dissolved oxygen (mg/L)	8.1	8.0	8.0	8.2	8.1	7.8
	Conductivity (µmhos/cm)	313		308		309	
	Alkalinity (mg $CaCO_3$/L)	58				62	
	Hardness (mg $CaCO_3$/L)	88			µ 08.09.25 N	88	
	Temperature (°C)	24.9	25.0	24.8	25.2	24.8	25.0
600 mg NaCl/L	pH (S.U.)	7.98	7.88	7.91	7.97	8.00	7.95
	Dissolved oxygen (mg/L)	8.1	7.9	8.0	8.1	8.1	7.8
	Conductivity (µmhos/cm)	1420		1440		1460	
	Temperature (°C)	25.0	24.8	25.0	24.9	24.9	25.1
800 mg NaCl/L	pH (S.U.)	7.99	7.88	7.92	7.98	8.02	7.93
	Dissolved oxygen (mg/L)	8.1	7.9	8.0	8.1	8.1	7.7
	Conductivity (µmhos/cm)	1770		1810		1840	
	Temperature (°C)	25.2	24.8	24.8	24.9	24.9	25.1
1000 mg NaCl/L	pH (S.U.)	7.99	7.87	7.92	7.97	8.02	7.93
	Dissolved oxygen (mg/L)	8.2	7.9	8.0	8.0	8.1	7.7
	Conductivity (µmhos/cm)	2140		2190		2210	
	Temperature (°C)	25.2	24.8	24.9	25.1	25.0	24.9
1200 mg NaCl/L	pH (S.U.)	7.99	7.87	7.92	7.97	8.03	7.93
	Dissolved oxygen (mg/L)	8.2	7.9	8.0	8.0	8.2	7.9
	Conductivity (µmhos/cm)	2480		2510		2570	
	Temperature (°C)	25.0	25.2	24.9	24.8	24.8	24.9
1400 mg NaCl/L	pH (S.U.)	7.99	7.87	7.91	7.98	8.03	7.93
	Dissolved oxygen (mg/L)	8.2	8.0	8.0	8.1	8.2	7.0
	Conductivity (µmhos/cm)	2860		2850		2950	
	Temperature (°C)	25.2	24.9	24.9	24.8	24.9	24.9
		Initial	Final	Initial	Final	Initial	Final

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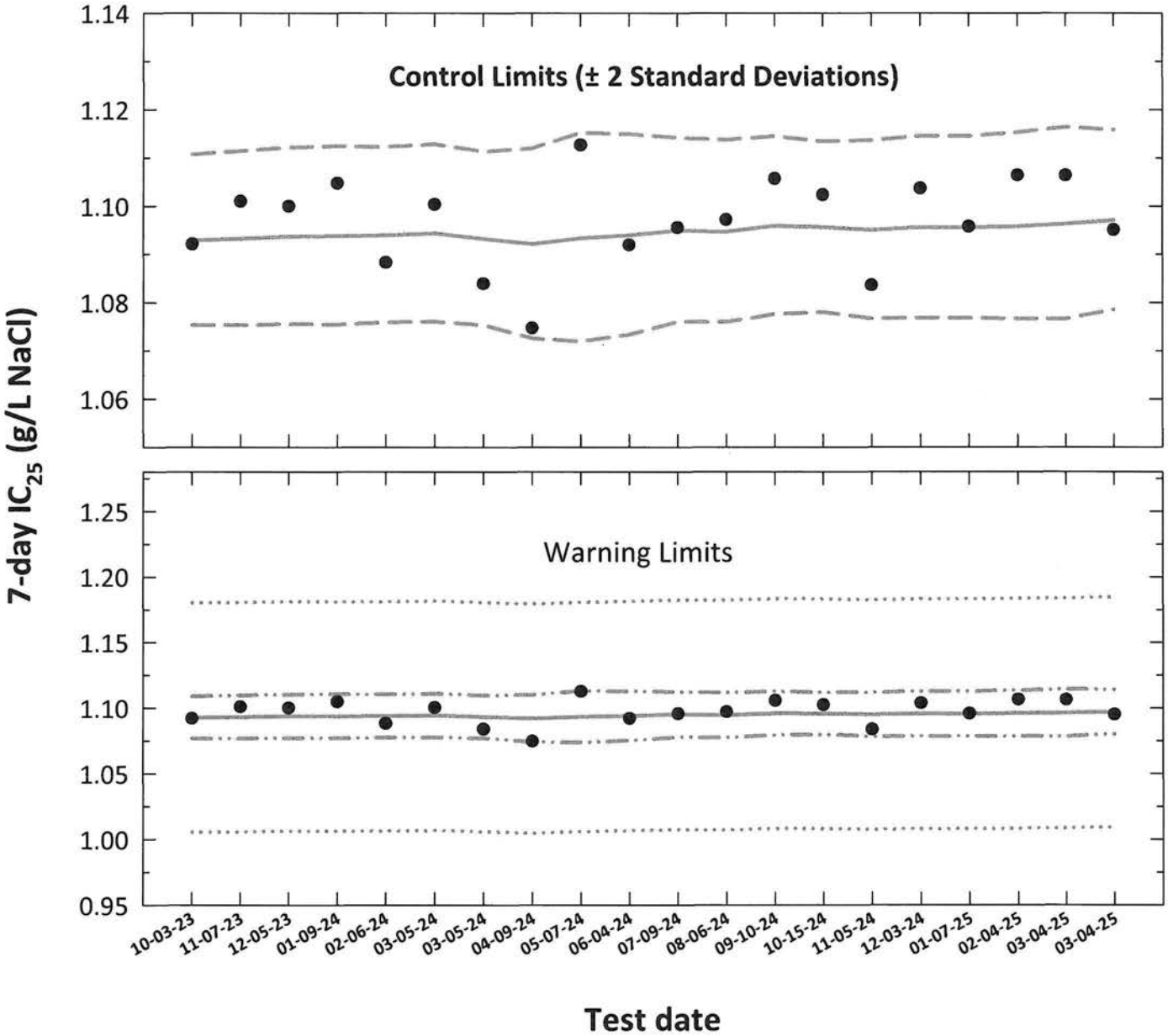
Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 308**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	K	K	K	K	K	Em	Em	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	8.09	7.98	8.01	8.11	8.11	7.97	7.90	7.99
	Dissolved oxygen (mg/L)	8.1	7.9	8.2	7.7	8.2	8.1	8.2	7.6
	Conductivity (µmhos/cm)	300		301		295		311	
	Alkalinity (mg $CaCO_3$/L)			62					
	Hardness (mg $CaCO_3$/L)		K 03-09-25 K	84					K 03-09-25 K
	Temperature (°C)	24.9	25.2	24.8	25.1	24.9	25.2	24.7	25.3
600 mg NaCl/L	pH (S.U.)	8.08	7.92	7.96	8.00	8.14	7.97	7.98	7.96
	Dissolved oxygen (mg/L)	8.1	7.7	8.1	7.7	8.2	8.1	8.2	7.6
	Conductivity (µmhos/cm)	1400		1400		1380		1440	
	Temperature (°C)	25.0	25.3	24.9	24.9	24.8	24.9	24.9	25.0
800 mg NaCl/L	pH (S.U.)	8.06	7.92	7.96	8.00	8.15	7.96	8.00	7.97
	Dissolved oxygen (mg/L)	8.2	7.6	8.1	7.7	8.2	8.0	8.2	7.6
	Conductivity (µmhos/cm)	1730		1730		1720		1820	
	Temperature (°C)	25.0	25.0	24.8	24.9	24.8	24.9	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	8.06	7.91	7.97	8.00	8.15	7.97	8.01	7.98
	Dissolved oxygen (mg/L)	8.2	7.6	8.1	7.7	8.2	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2070		2090		2080		2190	
	Temperature (°C)	25.0	25.0	24.8	25.0	25.0	25.1	24.8	25.0
1200 mg NaCl/L	pH (S.U.)	8.06	7.91	7.98	7.99	8.14	7.97	8.01	7.97
	Dissolved oxygen (mg/L)	8.2	7.6	8.1	7.7	8.2	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2400		2420		2420		2540	
	Temperature (°C)	25.0	24.9	25.0	25.0	25.0	24.8	24.8	25.2
1400 mg NaCl/L	pH (S.U.)	8.06	7.92	7.99	7.97	8.13	8.00	8.01	7.98
	Dissolved oxygen (mg/L)	8.2	7.6	8.1	7.7	8.3	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2750		2780		2740		2880	
	Temperature (°C)	24.9	24.9	25.0	25.0	24.9	25.1	24.9	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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	01-07-25	1.0958	0.0397	0.0396	0.0037	1.0955	1.0769	1.1145	1.0785	1.1126	1.0079	1.1832
18	02-04-25	1.1064	0.0439	0.0397	0.0038	1.0958	1.0767	1.1153	1.0783	1.1133	1.0082	1.1835
19	03-04-25	1.1064	0.0439	0.0400	0.0039	1.0964	1.0767	1.1165	1.0784	1.1144	1.0087	1.1841
20	03-04-25	1.0951	0.0394	0.0402	0.0037	1.0971	1.0786	1.1159	1.0802	1.1139	1.0093	1.1848

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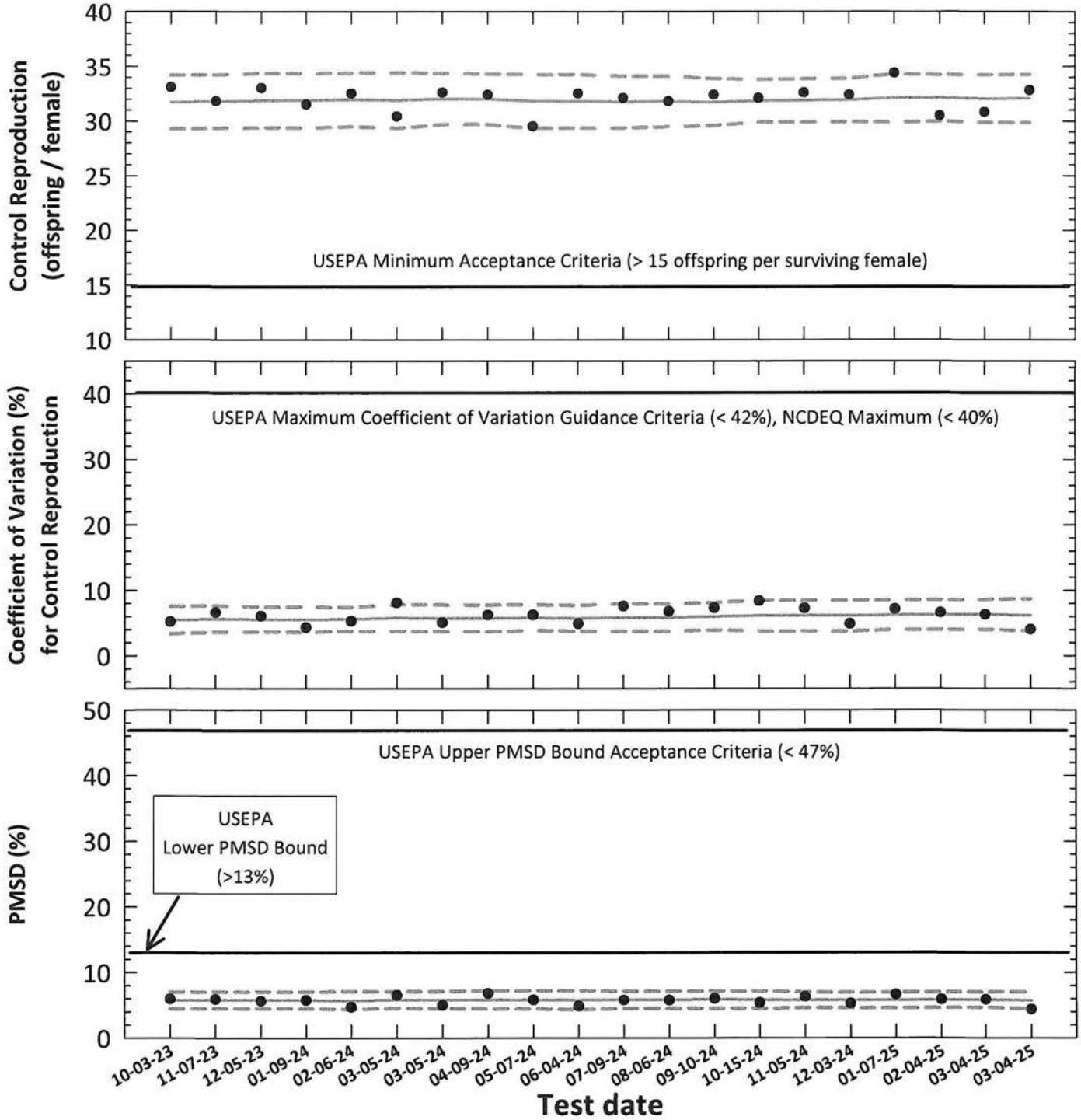
Ceriodaphnia dubia
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: In-house Culture



- **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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	01-07-25	100	34.4	7.1	2.280	6.6	32.1	29.9	34.3	6.2	4.0	8.5	5.7	4.5	7.0
18	02-04-25	100	30.5	6.6	1.782	5.8	32.1	30.0	34.3	6.3	4.0	8.5	5.8	4.6	7.0
19	03-04-25	100	30.8	6.3	1.797	5.8	32.0	29.9	34.2	6.3	4.0	8.6	5.8	4.6	7.0
20	03-04-25	100	32.8	4.0	1.430	4.4	32.1	29.9	34.3	6.2	3.7	8.7	5.7	4.4	7.0

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 #: 309 - New Algae Slant + YWT Lots

Dilution preparation information:						***Comments:***
NaCl Stock INSS number:		INSS 2356				
Stock preparation:		100 g NaCl/L: Dissolve 50 g NaCl in 500 mL deionized water.				
Dilution prep (mg/L)	600	800	1000	1200	1400	
Stock volume (mL)	9	12	15	18	21	
Diluent volume (mL)	1491	1488	1485	1482	1479	
Total volume (mL)	1500	1500	1500	1500	1500	

Test organism source:

Organism age:	< 24-hours old									
Date and times organisms were born between:	03-04-25 0530 TO 0800									
Culture board:	02-25-25 A									
Replicate number:	1	2	3	4	5	6	7	8	9	10
Culture board cup number:	4	6	8	12	13	14	15	17	18	19
Transfer vessel information:	pH (S.U.): 7.91 Temperature (°C): 25.1									
Average transfer volume (mL):	< 0.25 mL									

Test randomization and location:

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

Daily renewal:

Day	Date	Test initiation and feeding, renewal and feeding, or termination time	*Feeding Batches *Selenastrum*	*Feeding Batches YWT	MHSW batch used	Analyst
0	03-04-25	0819	02-24-25	02-28-25	02-26-25 A	JH
1	03-05-25	0620	↓	↓	↓	JH
2	03-06-25	0633	↓	↓	03-03-26 A	JH
3	03-07-25	0622	↓	↓	↓	JH
4	03-08-25	0633	↓	↓	03-06-25 A	JH
5	03-09-25	0621	↓	↓	↓	JH
6	03-10-25	0620	↓	↓	↓	JH
7	03-11-25	0625				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:	32.8	≥ 15.0 offspring/female	**ChV** (mg/L NaCl)	1095.5
% CV:	4.0%	< 40.0 %	**IC_{25}** (mg/L NaCl)	1095.1

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #: 309 - New Algae Slant + YWT Lots**

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	5	6	6	4	4	6	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	13	10	10	11	13	13	10	12
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	14	15	18	16	19	15	15	18	15
Total young produced		34	30	33	34	32	34	32	34	33	32
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:	32.8

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	6	4	5	5	5	6	5	5	5	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	11	10	12	12	10	13	13	13
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	14	19	17	16	17	13	19	17	15	14
Total young produced		32	33	33	31	34	31	34	35	33	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:	32.9
% Reduction from Control:	-0.3%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** **309 - New Algae Slant + YWT Lots**

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

Day		Replicate number 1	2	3	4	5	6	7	8	9	10
1	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
2	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
3	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
4	Young produced	4	5	5	5	6	4	4	6	6	6
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	12	10	12	12	12	12	11	11	10	11
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	18	14	14	16	13	17	19	16	16	16
Total young produced		34	29	31	33	31	33	34	33	32	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:	32.3
% Reduction from Control:	1.5%

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	5	5	4	4	4	4	5	5
	Adult mortality	L	L	L	L	L	L	L	L	L	L
5	Young produced	11	10	9	12	12	10	10	10	10	10
	Adult mortality	L	L	L	L	L	L	L	L	L	L
6	Young produced	0	0	0	0	0	0	0	0	0	0
	Adult mortality	L	L	L	L	L	L	L	L	L	L
7	Young produced	15	13	15	14	16	13	18	15	17	17
Total young produced		30	28	29	31	32	27	32	29	32	32
Final Adult Mortality		L	L	L	L	L	L	L	L	L	L

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	30.2
% Reduction from Control:	7.4%

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** **309 - New Algae Slant + YWT Lots**

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	18.5
% Reduction from Control:	43.6%

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

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

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

Concentration:	
% Mortality:	0%
Mean Offspring/Female:	2.1
% Reduction from Control:	93.6%

Environmental Testing Solutions, Inc.

Verification of *Ceriodaphnia* Reproduction Totals

Control

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	6	5	6	6	4	4	6	5	5	51
5	12	10	13	10	10	11	13	13	10	12	114
6	0	0	0	0	0	0	0	0	0	0	0
7	18	14	15	18	16	19	15	15	18	15	163
Total	**34**	**30**	**33**	**34**	**32**	**34**	**32**	**34**	**33**	**32**	**328**

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	5	5	4	4	4	4	5	5	45
5	11	10	9	12	12	10	10	10	10	10	104
6	0	0	0	0	0	0	0	0	0	0	0
7	15	13	15	14	16	13	18	15	17	17	153
Total	**30**	**28**	**29**	**31**	**32**	**27**	**32**	**29**	**32**	**32**	**302**

600 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	6	4	5	5	5	6	5	5	5	6	52
5	12	10	11	10	12	12	10	13	13	13	116
6	0	0	0	0	0	0	0	0	0	0	0
7	14	19	17	16	17	13	19	17	15	14	161
Total	**32**	**33**	**33**	**31**	**34**	**31**	**34**	**35**	**33**	**33**	**329**

1200 mg NaCl/L

Day	Replicate number										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	4	4	5	4	4	4	4	4	4	4	41
5	9	10	11	5	9	5	10	7	7	10	83
6	0	0	0	0	0	0	0	0	0	0	0
7	5	6	3	11	4	10	5	5	8	4	61
Total	**18**	**20**	**19**	**20**	**17**	**19**	**19**	**16**	**19**	**18**	**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	4	5	5	5	6	4	4	6	6	6	51
5	12	10	12	12	12	12	11	11	10	11	113
6	0	0	0	0	0	0	0	0	0	0	0
7	18	14	14	16	13	17	19	16	16	16	159
Total	**34**	**29**	**31**	**33**	**31**	**33**	**34**	**33**	**32**	**33**	**323**

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	1	3	1	2	4	1	1	2	2	2	19
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	1	0	0	0	0	1	0	0	0	0	2
Total	**2**	**3**	**1**	**2**	**4**	**2**	**1**	**2**	**2**	**2**	**21**

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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: 309, New Algae Slant, New YWT Consumables

Test dates: March 04-11, 2025

Concentration (mg/L NaCl)	Replicate number										Survival (%)	Average reproduction (offspring/female)	Coefficient of variation (%)	Percent reduction from control (%)
	1	2	3	4	5	6	7	8	9	10				
Control	34	30	33	34	32	34	32	34	33	32	**100**	**32.8**	**4.0**	**Not applicable**
600	32	33	33	31	34	31	34	35	33	33	**100**	**32.9**	**3.9**	**-0.3**
800	34	29	31	33	31	33	34	33	32	33	**100**	**32.3**	**4.9**	**1.5**
1000	30	28	29	31	32	27	32	29	32	32	**100**	**30.2**	**6.2**	**7.9**
1200	18	20	19	20	17	19	19	16	19	18	**100**	**18.5**	**6.9**	**43.6**
1400	2	3	1	2	4	2	1	2	2	2	**100**	**2.1**	**41.7**	**93.6**

Dunnett's MSD value: 1.430

PMSD: 4.4

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	3/4/2025	Test ID:	CdNaClCR	Sample ID:	REF-Ref Toxicant
End Date:	3/11/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	NACL-Sodium chloride
Sample Date:		Protocol:	FWCHR-EPA-821-R-02-013	Test Species:	CD-Ceriodaphnia dubia
Comments:					

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

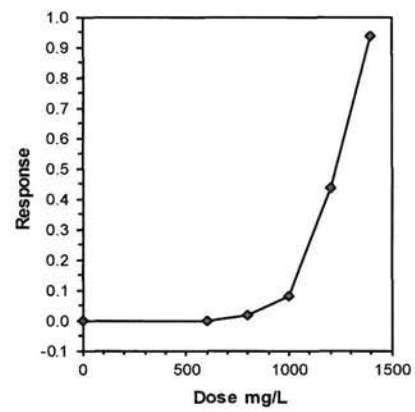
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	32.800	1.0000	32.800	30.000	34.000	4.014	10				32.850	1.0000
600	32.900	1.0030	32.900	31.000	35.000	3.911	10	-0.160	2.287	1.430	32.850	1.0000
800	32.300	0.9848	32.300	29.000	34.000	4.851	10	0.800	2.287	1.430	32.300	0.9833
*1000	30.200	0.9207	30.200	27.000	32.000	6.205	10	4.157	2.287	1.430	30.200	0.9193
*1200	18.500	0.5640	18.500	16.000	20.000	6.861	10	22.866	2.287	1.430	18.500	0.5632
*1400	2.100	0.0640	2.100	1.000	4.000	41.695	10	49.089	2.287	1.430	2.100	0.0639

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.81235	1.035	-0.5738	-0.1824
Bartlett's Test indicates equal variances (p = 0.39)	5.21392	15.0863		

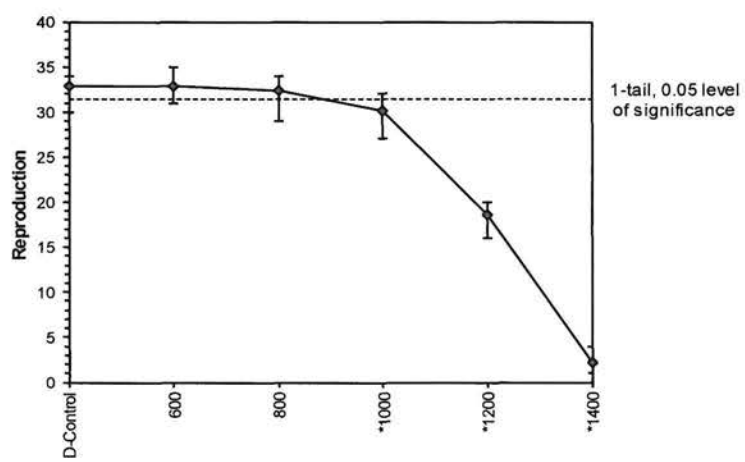
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	800	1000	894.427		1.43006	0.0436	1540	1.95556	0.0E+00	5, 54
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	904.048	51.6313	788.162	1002.22	-0.1292
IC10	1010.85	15.8377	963.171	1028.02	-1.9560
IC15	1038.93	8.62352	1019.53	1053.82	-0.1816
IC20	1067.01	7.55706	1050.84	1080.35	-0.0956
IC25	1095.09	6.72548	1081.6	1106.55	0.0354
IC40	1179.32	6.47106	1168.51	1191.96	0.3985
IC50	1225.3	4.31459	1217.52	1233.83	0.1717



Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** **309 - New Algae Slant + YWT Lots**

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.

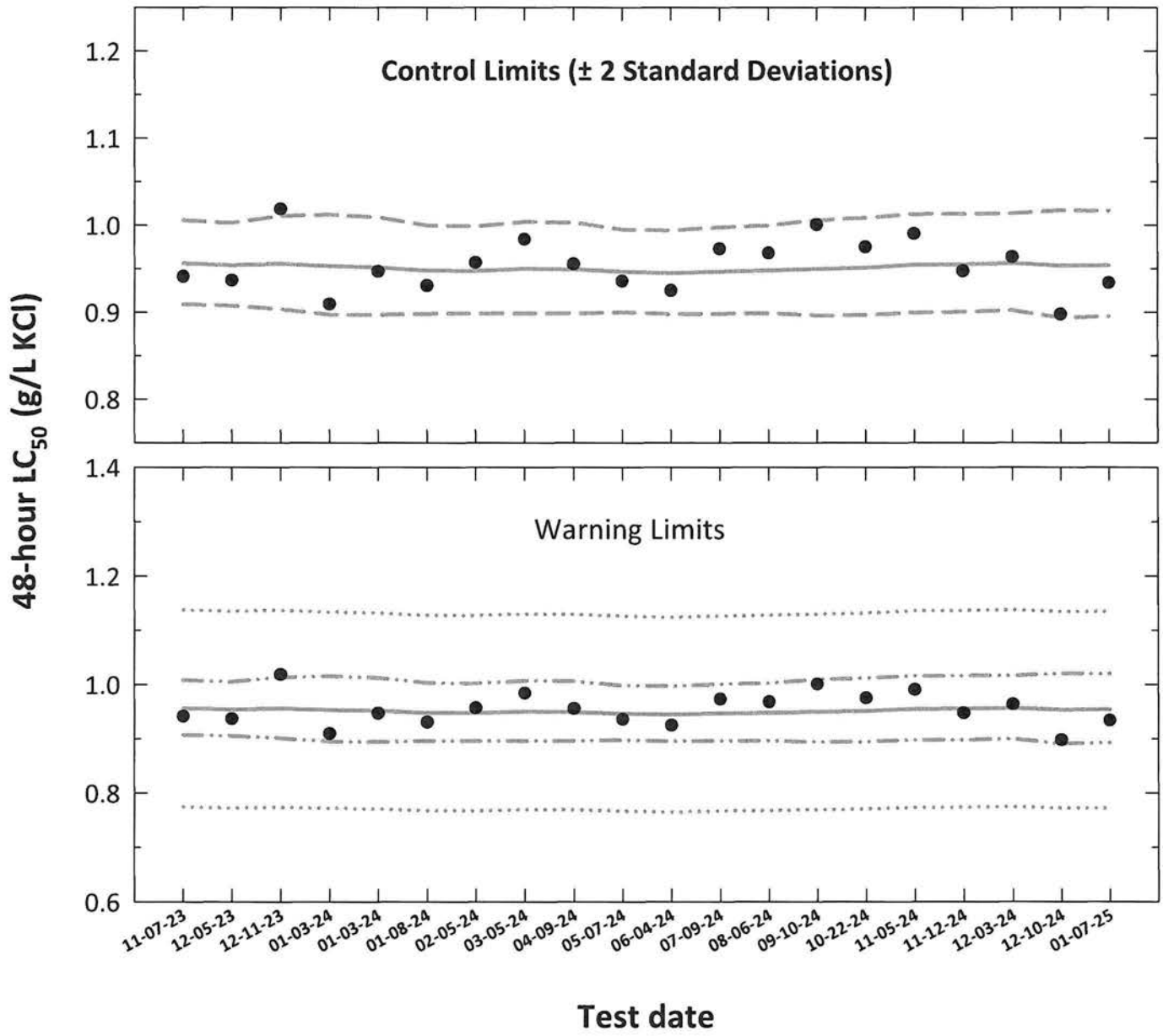
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	Em	Em	Em	Em	Em	M
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.96	7.83	7.86	7.94	7.93	7.94
	Dissolved oxygen (mg/L)	8.1	7.9	8.0	8.0	8.1	7.7
	Conductivity (µmhos/cm)	313		308		309	
	Alkalinity (mg $CaCO_3$/L)	58				62	
	Hardness (mg $CaCO_3$/L)	88			M 3-09-25 M	88	
	Temperature (°C)	24.9	25.3	24.8	25.1	24.9	25.2
600 mg NaCl/L	pH (S.U.)	7.98	7.84	7.91	7.92	8.00	7.89
	Dissolved oxygen (mg/L)	8.1	7.8	8.0	8.1	8.1	7.6
	Conductivity (µmhos/cm)	1420		1440		1460	
	Temperature (°C)	25.0	25.1	25.0	24.9	25.0	25.0
800 mg NaCl/L	pH (S.U.)	7.99	7.86	7.92	7.92	8.02	7.89
	Dissolved oxygen (mg/L)	8.1	7.8	8.0	8.0	8.1	7.6
	Conductivity (µmhos/cm)	1770		1810		1840	
	Temperature (°C)	25.1	25.0	25.0	24.9	24.8	25.2
1000 mg NaCl/L	pH (S.U.)	7.99	7.86	7.92	7.92	8.02	7.89
	Dissolved oxygen (mg/L)	8.2	7.8	8.0	8.0	8.1	7.6
	Conductivity (µmhos/cm)	2140		2190		2210	
	Temperature (°C)	25.2	25.2	24.8	25.2	24.8	25.2
1200 mg NaCl/L	pH (S.U.)	7.99	7.85	7.92	7.92	8.03	7.89
	Dissolved oxygen (mg/L)	8.2	7.8	8.0	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2480		2510		2570	
	Temperature (°C)	25.0	25.2	24.8	25.2	25.0	25.1
1400 mg NaCl/L	pH (S.U.)	7.99	7.86	7.91	7.95	8.03	7.90
	Dissolved oxygen (mg/L)	8.2	7.8	8.0	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2860		2850		2950	
	Temperature (°C)	25.2	24.9	24.8	24.9	25.0	25.2
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Ceriodaphnia dubia*** **CdNaClCR #:** **309 - New Algae Slant + YWT Lots**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	N	N	N	N	N	EM	EM	N
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	8.09	7.93	8.01	8.08	8.11	7.98	7.90	8.08
	Dissolved oxygen (mg/L)	8.1	7.5	8.2	7.6	8.2	8.0	8.2	7.6
	Conductivity (µmhos/cm)	300		301		295		311	
	Alkalinity (mg $CaCO_3$/L)			62					
	Hardness (mg $CaCO_3$/L)		N 03-09-25 N	84				N 03-09-25 N	
	Temperature (°C)	24.8	25.1	24.8	25.1	24.9	25.0	24.7	25.2
600 mg NaCl/L	pH (S.U.)	8.08	7.89	7.94	7.99	8.14	7.97	7.98	8.01
	Dissolved oxygen (mg/L)	8.1	7.5	8.1	7.6	8.2	8.0	8.2	7.6
	Conductivity (µmhos/cm)	1400		1400		1380		1440	
	Temperature (°C)	24.9	25.0	24.9	24.8	24.9	25.0	24.9	25.2
800 mg NaCl/L	pH (S.U.)	8.06	7.88	7.96	7.98	8.15	7.96	8.00	8.00
	Dissolved oxygen (mg/L)	8.2	7.5	8.1	7.6	8.2	8.0	8.2	7.6
	Conductivity (µmhos/cm)	1730		1730		1720		1820	
	Temperature (°C)	25.0	24.8	24.9	24.8	24.8	25.2	24.9	25.0
1000 mg NaCl/L	pH (S.U.)	8.06	7.87	7.97	7.98	8.15	7.97	8.01	8.00
	Dissolved oxygen (mg/L)	8.2	7.5	8.1	7.6	8.2	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2070		2090		2080		2190	
	Temperature (°C)	25.0	24.8	25.0	25.0	24.8	24.7	24.9	24.9
1200 mg NaCl/L	pH (S.U.)	8.06	7.89	7.98	7.98	8.14	7.98	8.01	7.99
	Dissolved oxygen (mg/L)	8.2	7.5	8.1	7.6	8.2	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2400		2420		2420		2540	
	Temperature (°C)	25.0	25.1	25.0	24.8	24.8	24.9	24.8	24.9
1400 mg NaCl/L	pH (S.U.)	8.06	7.89	7.99	7.97	8.13	7.99	8.01	8.00
	Dissolved oxygen (mg/L)	8.2	7.5	8.1	7.6	8.3	8.0	8.2	7.6
	Conductivity (µmhos/cm)	2750		2780		2740		2880	
	Temperature (°C)	25.0	24.9	24.9	25.0	24.9	24.9	24.9	24.9
		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	**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
2	**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
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**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
19	**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
20	**01-07-25**	**0.9340**	-0.0296	-0.0204	0.0138	0.9541	0.8955	1.0165	0.8927	1.0196	0.7728	1.1354

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 # 174

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

Stock solution INSS #: 2348

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	Em	Em	Em
Control, MHSW	pH (S.U.)	8.00	8.01	8.02
	Dissolved oxygen (mg/L)	7.6	7.6	7.7
	Conductivity (µmhos/cm)	316		
	Alkalinity (mg/L $CaCO_3$)	60		
	Hardness (mg/L $CaCO_3$)	87		
	Temperature (°C)	24.7	24.9	25.0
500 mg/L	pH (S.U.)	8.09	7.95	7.95
	Dissolved oxygen (mg/L)	7.8	7.6	7.6
	Conductivity (µmhos/cm)	1220		
	Temperature (°C)	25.0	25.1	24.7
750 mg/L	pH (S.U.)	8.08	7.97	7.91
	Dissolved oxygen (mg/L)	7.8	7.5	7.5
	Conductivity (µmhos/cm)	1630		
	Temperature (°C)	25.0	25.1	24.7
1000 mg/L	pH (S.U.)	8.08	7.93	7.92
	Dissolved oxygen (mg/L)	7.9	7.5	7.4
	Conductivity (µmhos/cm)	2080		
	Temperature (°C)	24.8	24.8	24.6
1250 mg/L	pH (S.U.)	8.06	7.89	7.93
	Dissolved oxygen (mg/L)	7.9	7.4	7.4
	Conductivity (µmhos/cm)	2500		
	Temperature (°C)	24.8	24.8	24.7
1500 mg/L	pH (S.U.)	8.08	7.87	
	Dissolved oxygen (mg/L)	7.9	7.7	
	Conductivity (µmhos/cm)	2910		
	Temperature (°C)	24.9	25.0	

8.08.15

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by t analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identifi 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 # 174

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	01.07.25	* 0505	H	0705	H	1E	PINK	01.02.25B
24	01.08.25			0700	H			
48 Termination	01.09.25			0700	H			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	12.25.24
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	12.31.24 1126 TO 01.01.25 0515
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.37
	Temperature (°C): 24.0°C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	6 4d	7 3d	0 10d	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	9 1d	9 1d	5 1d	3 4d	0	0 1d	0	0
Mean Survival	100%		100%		90%		40%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	866.3
Upper 95% confidence limit (mg KCl/L)	998.9
48-hour LC_{50} (mg KCl/L)	934.0

Comments:

Test Reviewed by: H

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	1/7/2025	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	1/9/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

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

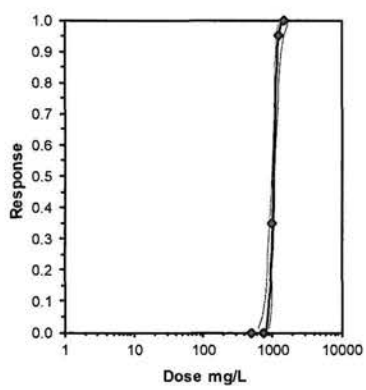
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1748	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1748	0	20
*1000	0.6500	0.6500	0.9386	0.8861	0.9912	7.916	2	7.720	2.850	0.1748	7	20
*1250	0.0500	0.0500	0.2403	0.1588	0.3218	47.963	2	19.109	2.850	0.1748	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				

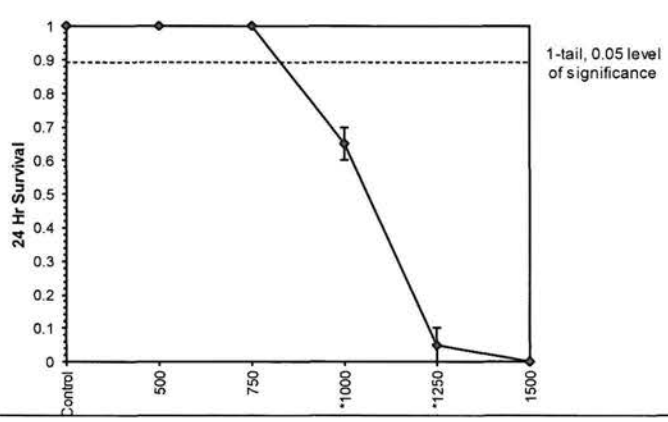
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08218	0.08429	0.5279	0.00376	2.5E-05	4, 5
Treatments vs D-Control										

					Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	21.4313	5.18429	11.2701	31.5925	0	0.03557	7.81472	0.99823	3.01869	0.04666	3
Intercept	-59.694	15.6806	-90.428	-28.96							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	813.086	634.929	894.023
EC05	3.355	874.854	727.146	942.96
EC10	3.718	909.677	780.747	971.27
EC15	3.964	933.952	818.455	991.661
EC20	4.158	953.707	849.118	1008.89
EC25	4.326	970.987	875.727	1024.62
EC40	4.747	1015.93	942.342	1070.09
EC50	5.000	1043.96	980.316	1103.47
EC60	5.253	1072.77	1015.22	1143.05
EC75	5.674	1122.43	1065.8	1223.63
EC80	5.842	1142.76	1083.84	1260.31
EC85	6.036	1166.93	1103.92	1306.04
EC90	6.282	1198.07	1128.22	1367.76
EC95	6.645	1245.76	1163.17	1467.22
EC99	7.326	1340.4	1227.9	1678.87



Dose-Response Plot



Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	1/7/2025	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	1/9/2025	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	0.3000
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	0.1854	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	2.505	2.850	0.1854	2	20
*1000	0.4000	0.4000	0.6825	0.5796	0.7854	21.317	2	11.212	2.850	0.1854	12	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	19.261	2.850	0.1854	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

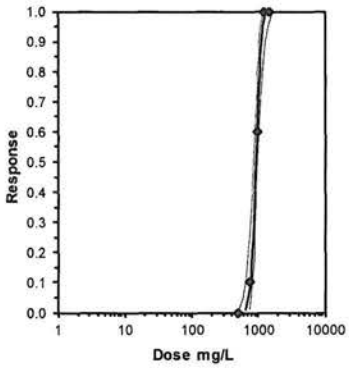
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08888	0.09116	0.60426	0.00423	2.4E-05	4, 5
Treatments vs D-Control										

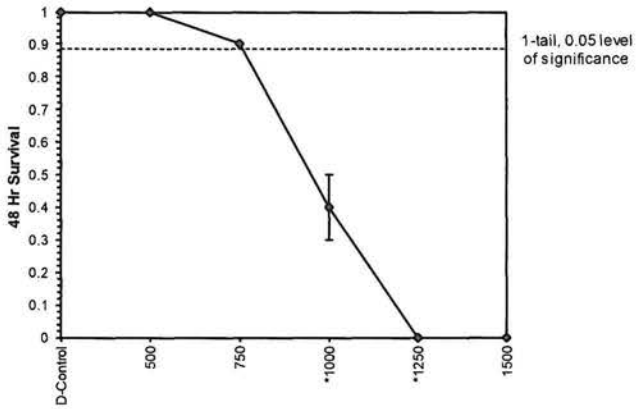
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.2157	2.93091	9.47108	20.9602	0	1.27858	7.81472	0.73423	2.97036	0.06572	5
Intercept	-40.196	8.73997	-57.326	-23.066							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	656.859	515.264	738.8
EC05	3.355	728.218	605.237	800.018
EC10	3.718	769.375	658.53	835.876
EC15	3.964	798.45	696.463	861.777
EC20	4.158	822.339	727.603	883.612
EC25	4.326	843.403	754.882	903.436
EC40	4.747	898.904	824.724	959.46
EC50	5.000	934.037	866.267	998.896
EC60	5.253	970.542	906.397	1043.97
EC75	5.674	1034.41	968.974	1133.07
EC80	5.842	1060.91	992.55	1173.37
EC85	6.036	1092.65	1019.42	1223.77
EC90	6.282	1133.94	1052.65	1292.24
EC95	6.645	1198.03	1101.51	1403.88
EC99	7.326	1328.18	1194.59	1646.53



Dose-Response Plot



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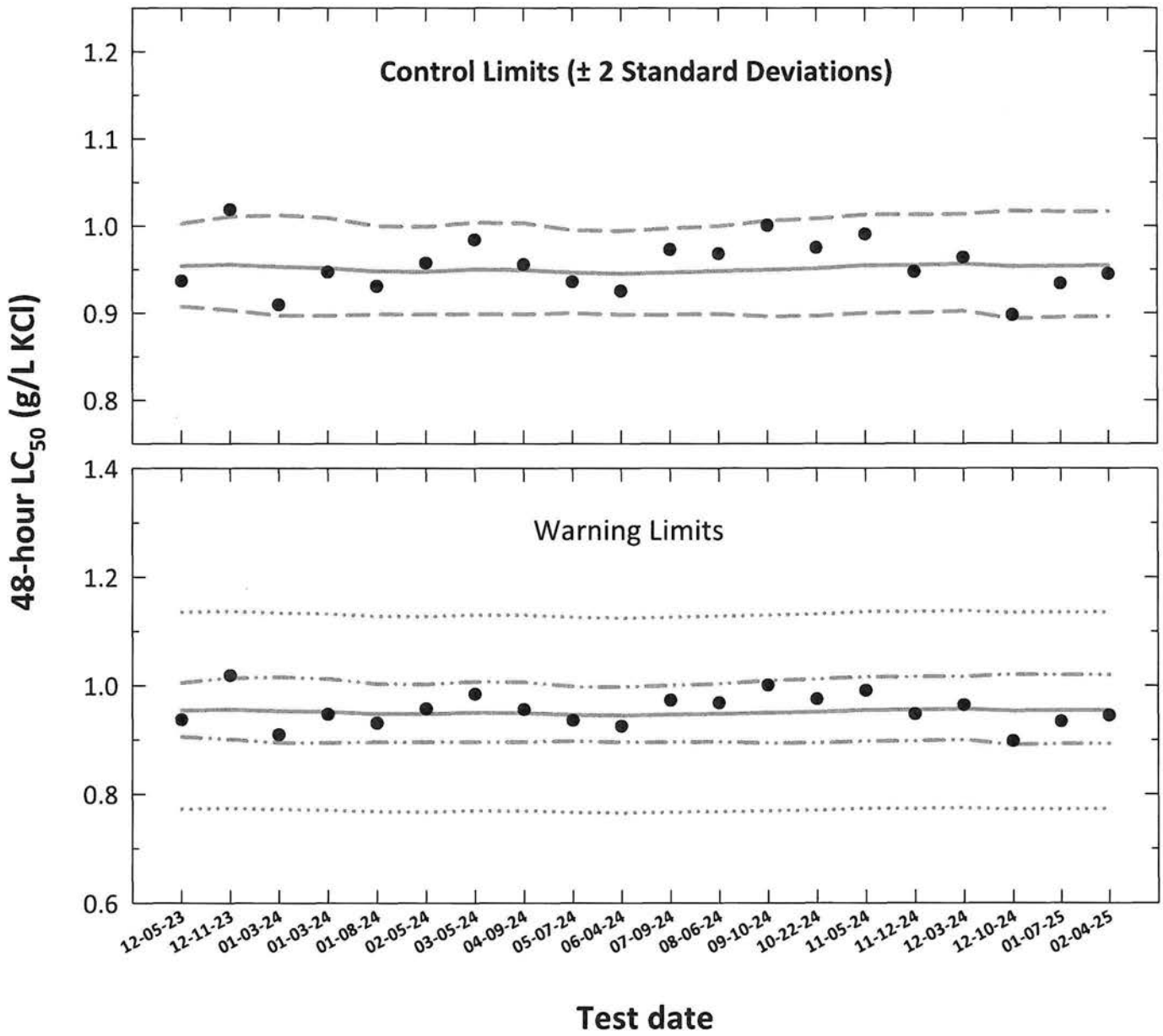
Pimephales promelas
Acute Reference Toxicant Control Chart
Source: In-house Culture



- ● **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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	01-07-25	**0.9340**	-0.0296	-0.0204	0.0138	0.9541	0.8955	1.0165	0.8927	1.0196	0.7728	1.1354
20	02-04-25	**0.9448**	-0.0247	-0.0203	0.0137	0.9543	0.8958	1.0166	0.8930	1.0196	0.7730	1.1356

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 # 175

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

Stock solution INSS #: 2348

Chemical Analyses:

		Hours		
		0	24	48
Concentration	Analyst	VL	EM	EM
Control, MHSW	pH (S.U.)	8.05	7.75	7.90
	Dissolved oxygen (mg/L)	8.0	7.8	7.8
	Conductivity (µmhos/cm)	300		
	Alkalinity (mg/L $CaCO_3$)	59		
	Hardness (mg/L $CaCO_3$)	90		
	Temperature (°C)	24.6	25.0	25.1
500 mg/L	pH (S.U.)	8.02	7.78	7.87
	Dissolved oxygen (mg/L)	8.0	7.8	7.8
	Conductivity (µmhos/cm)	1170		
	Temperature (°C)	24.7	25.2	24.8
750 mg/L	pH (S.U.)	7.99	7.79	7.83
	Dissolved oxygen (mg/L)	8.0	7.8	7.7
	Conductivity (µmhos/cm)	1620		
	Temperature (°C)	24.7	25.2	25.2
1000 mg/L	pH (S.U.)	7.98	7.76	7.83
	Dissolved oxygen (mg/L)	8.0	7.8	7.7
	Conductivity (µmhos/cm)	2000		
	Temperature (°C)	24.7	24.9	24.9
1250 mg/L	pH (S.U.)	7.97	7.76	7.82
	Dissolved oxygen (mg/L)	8.0	7.8	7.6
	Conductivity (µmhos/cm)	2510		
	Temperature (°C)	24.6	24.9	24.8
1500 mg/L	pH (S.U.)	7.98	7.74	
	Dissolved oxygen (mg/L)	8.0	7.7	
	Conductivity (µmhos/cm)	3930		KO2-05-25
	Temperature (°C)	24.6	25.2	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by tl analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identifi 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	13066466

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 # 175

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	02-04-25	* 0500	JK	0700	JK	1F	PINK	01-26-25 B
24	02-05-25			0650	JK			
48 Termination	02-06-25			0652	JK			

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

Test Organism Information:

Organism Source:	In-house culture
Spawning date:	01-22-25
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	01-28-25 1508 TO 01-29-25 0840
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 8.45
	Temperature (°C): 24.0°C

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

Survival Data (number of living organisms):

Hours	Control		500 mg/L		750 mg/L		1000 mg/L		1250 mg/L		1500 mg/L	
	Replicate		Replicate		Replicate		Replicate		Replicate		Replicate	
	A	B	C	D	E	F	G	H	I	J	K	L
0 Initiation	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	10	10	10	10	6 4d	5 5d	2 8d	2 8d	0 10d	0 10d
48 Termination	10	10	10	10	9 1d	9 1d	5 1d	4 1d	0 2d	0 2d	0	0
Mean Survival	100%		100%		90%		45%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	875.9
Upper 95% confidence limit (mg KCl/L)	1010.5
48-hour LC_{50} (mg KCl/L)	944.8

Comments:

Test Reviewed by: JK

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	2/4/2025	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	2/6/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

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

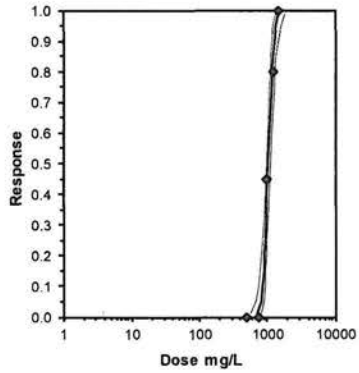
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
750	1.0000	1.0000	1.4120	1.4120	1.4120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

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

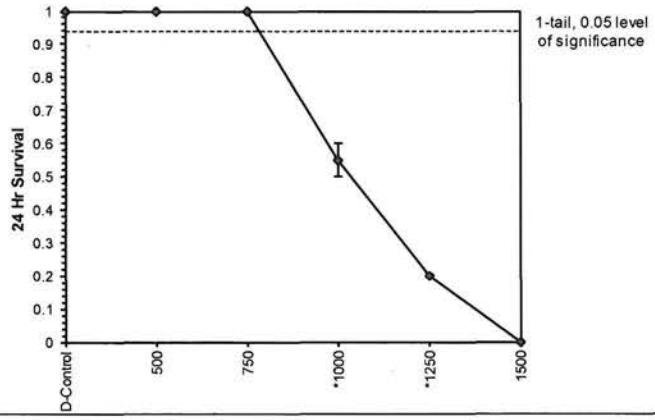
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.03598	0.0369	0.3833	0.00101	2.2E-06	4, 5
Treatments vs D-Control										

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

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



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	2/4/2025	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	2/6/2025	Lab ID.	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

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

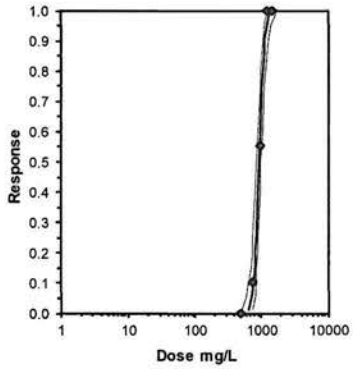
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	5.119	2.850	0.0907	2	20
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	21.263	2.850	0.0907	11	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	39.364	2.850	0.0907	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

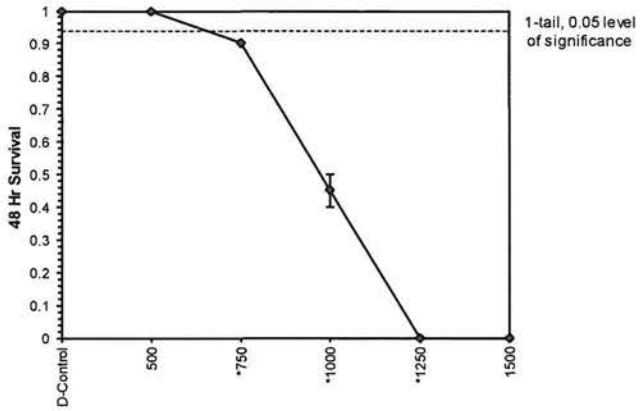
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.03598	0.0369	0.58958	0.00101	7.4E-07	4, 5
Treatments vs D-Control										

			Maximum Likelihood-Probit								
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.932	2.848	9.34991	20.5141	0	1.85598	7.81472	0.60283	2.97534	0.06697	5
Intercept	-39.428	8.50889	-56.105	-22.75							
TSCR											

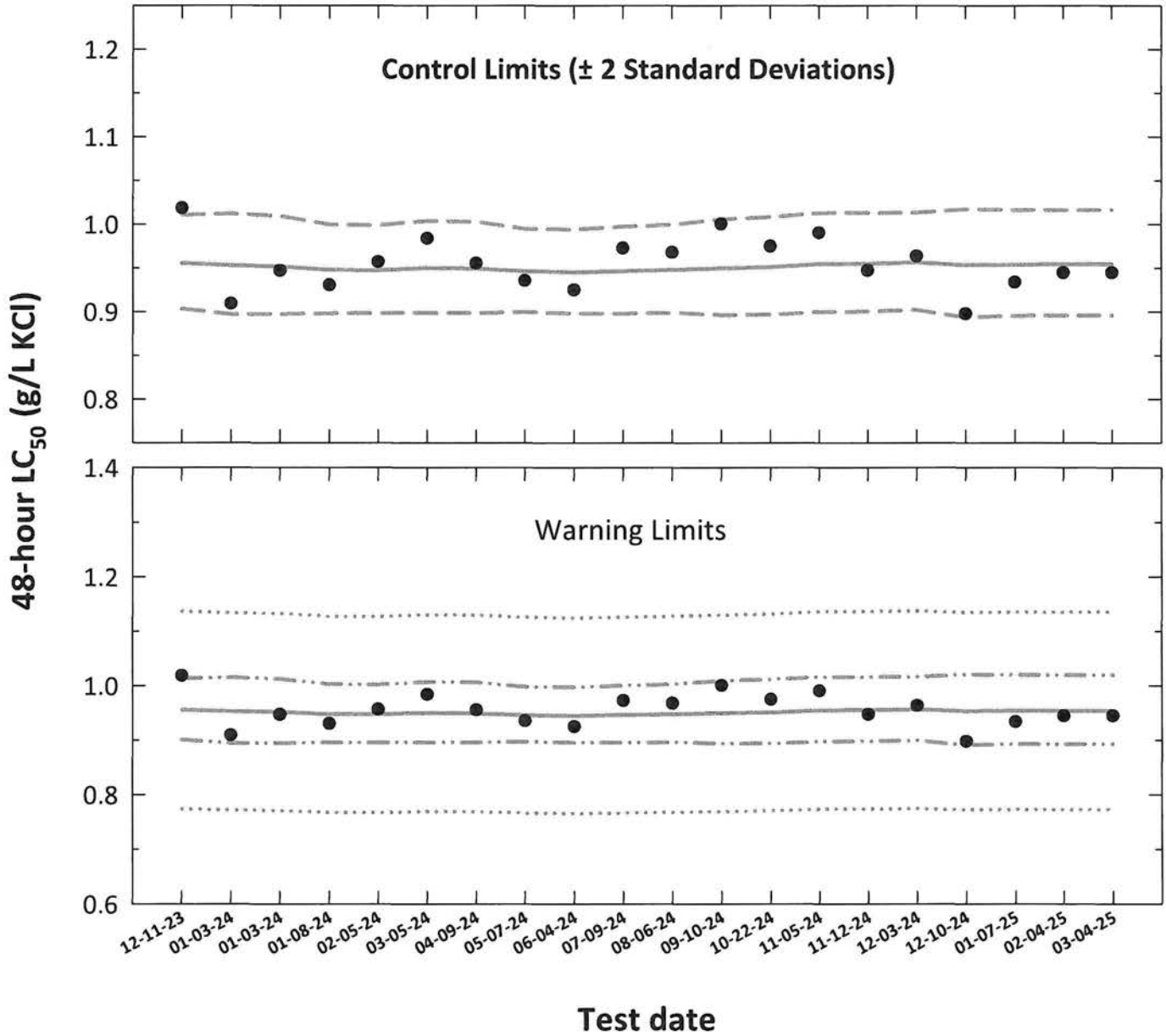
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	660.006	517.08	743.411
EC05	3.355	733.142	608.693	806.301
EC10	3.718	775.387	663.065	843.151
EC15	3.964	805.256	701.817	869.766
EC20	4.158	829.814	733.664	892.198
EC25	4.326	851.478	761.59	912.556
EC40	4.747	908.61	833.219	970.049
EC50	5.000	944.81	875.925	1010.49
EC60	5.253	982.451	917.251	1056.71
EC75	5.674	1048.37	981.814	1148.12
EC80	5.842	1075.74	1006.17	1189.51
EC85	6.036	1108.55	1033.95	1241.29
EC90	6.282	1151.25	1068.33	1311.69
EC95	6.645	1217.59	1118.94	1426.58
EC99	7.326	1352.51	1215.51	1676.69



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	**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
2	**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
3	**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
4	**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
5	**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
6	**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
7	**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
8	**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
9	**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
10	**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
11	**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
12	**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
13	**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
14	**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
15	**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
16	**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
17	**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
18	**01-07-25**	**0.9340**	-0.0296	-0.0204	0.0138	0.9541	0.8955	1.0165	0.8927	1.0196	0.7728	1.1354
19	**02-04-25**	**0.9448**	-0.0247	-0.0203	0.0137	0.9543	0.8958	1.0166	0.8930	1.0196	0.7730	1.1356
20	**03-04-25**	**0.9448**	-0.0247	-0.0203	0.0137	0.9543	0.8958	1.0166	0.8930	1.0196	0.7730	1.1356

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 # 176

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 #: 2357

Chemical Analyses:

Concentration		Hours 0	24	48
	Analyst	Em	Em	Em
Control, MHSW	pH (S.U.)	7.96	7.88	7.99
	Dissolved oxygen (mg/L)	8.1	7.9	7.8
	Conductivity (µmhos/cm)	313		
	Alkalinity (mg/L $CaCO_3$)	58		
	Hardness (mg/L $CaCO_3$)	88		
	Temperature (°C)	24.5	24.9	25.1
500 mg/L	pH (S.U.)	8.01	7.85	7.94
	Dissolved oxygen (mg/L)	8.2	7.8	7.7
	Conductivity (µmhos/cm)	1170		
	Temperature (°C)	24.8	25.2	24.8
750 mg/L	pH (S.U.)	8.01	7.83	7.93
	Dissolved oxygen (mg/L)	8.2	7.8	7.7
	Conductivity (µmhos/cm)	1580		
	Temperature (°C)	24.6	24.8	24.8
1000 mg/L	pH (S.U.)	8.00	7.77	7.89
	Dissolved oxygen (mg/L)	8.2	7.8	7.7
	Conductivity (µmhos/cm)	1960		
	Temperature (°C)	24.6	24.8	24.6
1250 mg/L	pH (S.U.)	8.00	7.79	7.94
	Dissolved oxygen (mg/L)	8.2	7.8	7.6
	Conductivity (µmhos/cm)	2410		
	Temperature (°C)	24.4	24.7	24.9
1500 mg/L	pH (S.U.)	8.00	7.75	
	Dissolved oxygen (mg/L)	8.2	7.7	
	Conductivity (µmhos/cm)	2810		
	Temperature (°C)	24.6	24.9	

*Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurements only. Temperatures performed at the time of test initiation or termination by 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	130404685

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 # 176

Hours	Date	Feeding		Test Initiation or Termination		Location	Randomizing	MHSW Batch
		Time	Analyst	Time	Analyst	Incubator/Shelf	Template	
0 Initiation	03-04-25	* 0500	JH	0700	JH	1E	PINK	02-26-25A
24	03-05-25			0700	JH			
48 Termination	03-06-25			0655	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:	02-19-25
Age (1 to 14 days old):	6 TO 7 DAYS
Hatch date and times:	02-25-25 1323 TO 02-26-25 0833
Average transfer volume:	< 0.25 mL
Transfer bowl information:	pH (S.U.): 7.96
	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	9 1d	9 1d	9 1d 4	5 5d	0 10d	1 9d	0 10d	0 10d
48 Termination	10	10	10	10	9	9	4	5	0	0 1d	0	0
Mean Survival	100%		100%		90%		45%		0%		0%	

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

Statistics:

Method	PROBIT
Lower 95% confidence limit (mg KCl/L)	875.9
Upper 95% confidence limit (mg KCl/L)	1010.5
48-hour LC_{50} (mg KCl/L)	944.8

Comments:

Test Reviewed by: JH

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-48 Hr Survival

Start Date:	3/4/2025	Test ID:	PpKClAC	Sample ID:	REF-Ref Toxicant
End Date:	3/6/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

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

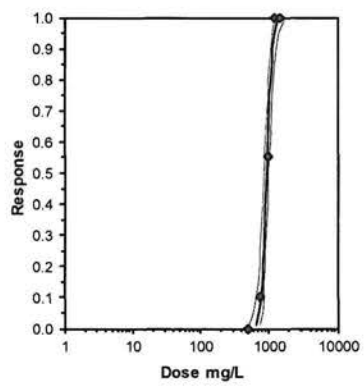
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.0907	0	20
*750	0.9000	0.9000	1.2490	1.2490	1.2490	0.000	2	5.119	2.850	0.0907	2	20
*1000	0.4500	0.4500	0.7351	0.6847	0.7854	9.685	2	21.263	2.850	0.0907	11	20
*1250	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2	39.364	2.850	0.0907	20	20
1500	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	2				20	20

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

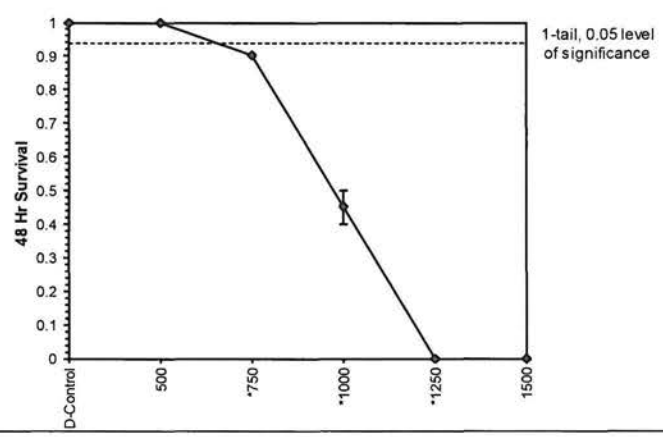
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	500	750	612.372		0.03598	0.0369	0.58958	0.00101	7.4E-07	4, 5
Treatments vs D-Control										

			Maximum Likelihood-Probit								
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.932	2.848	9.34991	20.5141	0	1.85598	7.81472	0.60283	2.97534	0.06697	5
Intercept	-39.428	8.50889	-56.105	-22.75							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	660.006	517.08	743.411
EC05	3.355	733.142	608.693	806.301
EC10	3.718	775.387	663.065	843.151
EC15	3.964	805.256	701.817	869.766
EC20	4.158	829.814	733.664	892.198
EC25	4.326	851.478	761.59	912.556
EC40	4.747	908.61	833.219	970.049
EC50	5.000	944.81	875.925	1010.49
EC60	5.253	982.451	917.251	1056.71
EC75	5.674	1048.37	981.814	1148.12
EC80	5.842	1075.74	1006.17	1189.51
EC85	6.036	1108.55	1033.95	1241.29
EC90	6.282	1151.25	1068.33	1311.69
EC95	6.645	1217.59	1118.94	1426.58
EC99	7.326	1352.51	1215.51	1676.69



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Acute Fathead Minnow Test-24 Hr Survival

Start Date:	3/4/2025	Test ID:	PpKCIAC	Sample ID:	REF-Ref Toxicant
End Date:	3/6/2025	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:					

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

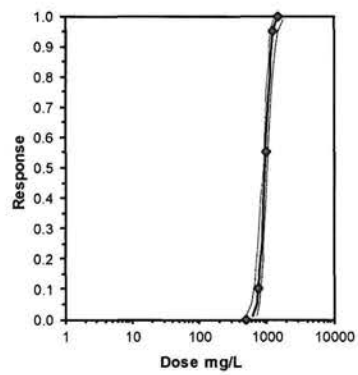
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2				0	20
500	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	2	0.000	2.850	0.1726	0	20
750	0.9000	0.9000	1.2490	1.2490	1.2490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

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

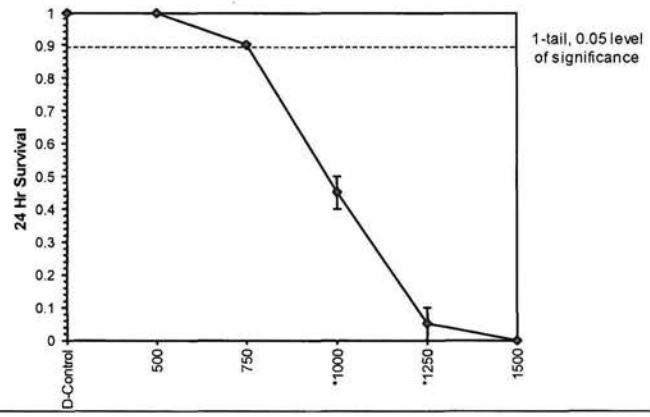
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	750	1000	866.025		0.08088	0.08295	0.52423	0.00367	2.4E-05	4, 5
Treatments vs D-Control										

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

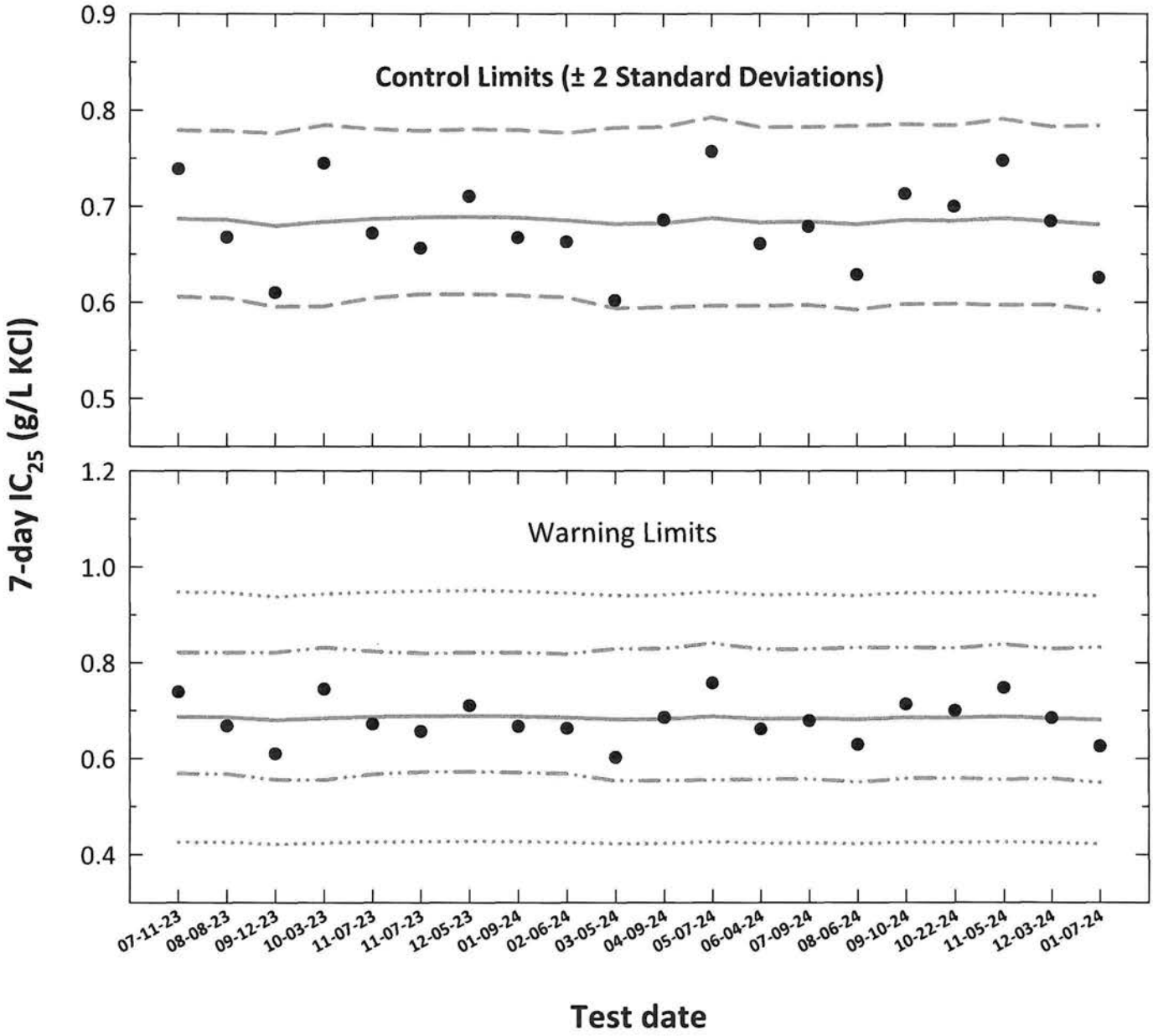
Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	639.176	493.334	727.802
EC05	3.355	719.447	590.487	797.807
EC10	3.718	766.282	648.953	839.028
EC15	3.964	799.591	690.984	868.855
EC20	4.158	827.094	725.754	894.014
EC25	4.326	851.442	756.417	916.854
EC40	4.747	916.021	835.854	981.348
EC50	5.000	957.206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



Dose-Response Plot



Pimephales promelas
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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	01-07-24	**0.6255**	-0.2038	-0.1668	0.0305	0.6811	0.5917	0.7840	0.5499	0.8321	0.4223	0.9399

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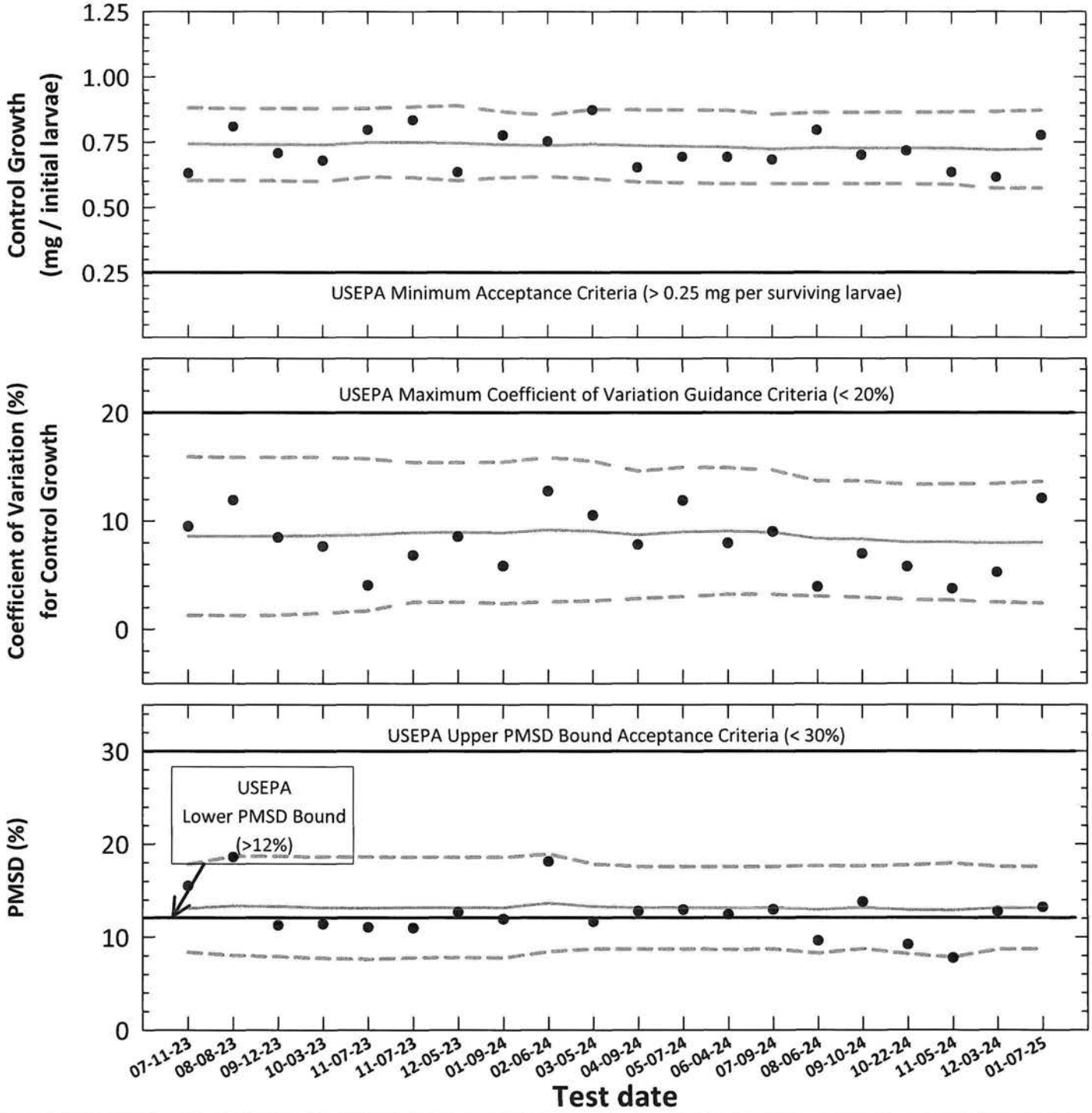
Pimephales promelas
Chronic Reference Toxicant Testing, Test Acceptability Criteria
Organism Source: In-house Culture



- **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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	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
20	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	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: **123**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2348				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	12-31-24	*Artemia* CHM number:	CHM1294
Hatch dates and times:	01-06-25 1425 TO 01-07-25 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.37 S.U. Temperature = 24.0 °C	Date / Time in oven:	01-14-25 0600
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	01-15-25 0600
		*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	01-07-25	0505	JK	1430	JK	0711	JK	01-02-25 B
1	01-08-25	0500	JK	1100	JK	0710	JK	↓
2	01-09-25	0500	JK	1215	JK	0710	JK	01-06-25
3	01-10-25	0500	JK	1100	JK	0700	JK	↓
4	01-11-25	0600	JK	1300	JK	0800	JK	01-07-25
5	01-12-25	0600	JK	1200	JK	0800	JK	↓
6	01-13-25	0500	JK	1230	JK	0735	JK	
7	01-14-25					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)	758.5
Average weight per initial larvae:	0.776		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.776	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	625.5

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **123**

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: LIGHT PINK Analyst: JI Date: 12-17-24	14.39	12.70	13.37	13.99	13.36	12.58	14.93	12.07	15.10	13.71	12.88	13.75
*B = Pan + Larvae weight (mg) Analyst: EM Date: 01-20-25	22.70	19.22	20.94	22.63	22.39	21.35	24.19	21.04	23.04	20.50	21.23	21.82
C = Larvae weight (mg) = B – A Analyst: JI	8.31	6.52	7.57	8.64	9.03	8.77	9.26	8.37	7.94	6.79	8.35	8.07
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JI	0.831	0.652	0.757	0.864	0.903	0.877	0.926	0.837	0.794	0.679	0.835	0.807
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.776				0.886		-14.1%		0.779		-0.4%	

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **123**

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	9 d	10	9 d
2	10	10	10	10	8 2d	7 3d	8 2d	8 2d	7 3d	6 3d	6 4d	6 3d
3	10	9 d	10	9 d ~~10~~	6 2d ~~8~~	7 ~~7~~	7 d ~~8~~	6 2d ~~8~~ sk 01-10-25	5 2d	3 3d	3 3d	4 2d
4	10	9	10	9	6	7	7	6	3 2d	~~2~~ 3	3	~~3~~ 4
5	10	9	10	8 d	3 3d	4 3d	4 3d	4 2d	3	3	3	3 d
6	10	9	10	8	3	4	4	4	3	3	3	3
7	10	9	10	8	3	4	4	4	3	3	2 m	3
*A = Pan weight (mg) Tray color code: Light pink Analyst: XL Date: 12-17-24	12.34	14.22	13.57	15.39	13.16	14.32	12.68	12.53	11.34	13.75	11.02	13.85
*B = Pan + Larvae weight (mg) Analyst: Em Date: 01-20-25	19.50	20.77	20.76	22.15	15.36	17.61	14.93	15.79	13.60	15.95	12.88	15.84
C = Larvae weight (mg) = B – A Analyst: JL	7.16	6.55	7.19	6.76	2.20	3.29	2.85	3.26	2.26	2.20	1.86	1.99
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.716	0.655	0.719	0.676	0.220	0.329	0.285	0.326	0.226	0.220	0.186	0.199
Average weight per initial number of larvae (mg)	0.692				0.290				0.208			
Percent reduction from control (%)		10.9%				62.6%				73.2%		

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **123**

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	8 2d
2	5 3d	3 5d	3 5d	3 5d
3	2 3d	2 1d	2 1d	2 1d
4	2	1 1d	2	1 1d
5	1 1d	1	1 1d	1
6	1	0 1d	1	1
7	0 1d	0	1	1
*A = Pan weight (mg) Tray color code: Light pink Analyst: XC Date: 12-17-24	12.48	13.05	15.28	13.66
*B = Pan + Larvae weight (mg) Analyst: EM Date: 01-20-25			15.49	14.19
C = Larvae weight (mg) = B – A Analyst: JI		01-21-25 K	0.21	0.53
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JI	0	0	0.021	0.053
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.019		97.6%	

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

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

Comments:

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: 123

Test dates: January 07-14, 2025

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	14.39	22.70	8.31	0.831	0.776	12.1	0.831	100.0	0.776	12.1	Not applicable
	B	10	10	12.70	19.22	6.52	0.652			0.652				
	C	10	10	13.37	20.94	7.57	0.757			0.757				
	D	10	10	13.99	22.63	8.64	0.864			0.864				
300	E	10	10	13.36	22.39	9.03	0.903	0.886	4.3	0.903	100.0	0.886	4.3	-14.1
	F	10	10	12.58	21.35	8.77	0.877			0.877				
	G	10	10	14.93	24.19	9.26	0.926			0.926				
	H	10	10	12.67	21.04	8.37	0.837			0.837				
450	I	10	10	15.10	23.04	7.94	0.794	0.779	8.8	0.794	100.0	0.779	8.8	-0.4
	J	10	10	13.71	20.50	6.79	0.679			0.679				
	K	10	10	12.88	21.23	8.35	0.835			0.835				
	L	10	10	13.75	21.82	8.07	0.807			0.807				
600	M	10	10	12.34	19.50	7.16	0.716	0.752	8.3	0.716	92.5	0.692	4.5	10.9
	N	10	9	14.22	20.77	6.55	0.728			0.655				
	O	10	10	13.57	20.76	7.19	0.719			0.719				
	P	10	8	15.39	22.15	6.76	0.845			0.676				
750	Q	10	3	13.16	15.36	2.20	0.733	0.771	7.3	0.220	37.5	0.290	17.5	62.6
	R	10	4	14.32	17.61	3.29	0.823			0.329				
	S	10	4	12.08	14.93	2.85	0.713			0.285				
	T	10	4	12.53	15.79	3.26	0.815			0.326				
900	U	10	3	11.34	13.60	2.26	0.753	0.770	14.7	0.226	27.5	0.208	8.9	73.2
	V	10	3	13.75	15.95	2.20	0.733			0.220				
	W	10	2	11.02	12.88	1.86	0.930			0.186				
	X	10	3	13.85	15.84	1.99	0.663			0.199				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.370	61.2	0.000	5.0	0.019	135.4	97.6
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	1	15.28	15.49	0.21	0.210			0.021				
	BB	10	1	13.66	14.19	0.53	0.530			0.053				

Dunnett's MSD value: 0.1024 MSD = Minimum Significant Difference

PMSD: 13.2 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 Jeff Swanner

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	1/7/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	1/14/2025	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	0.9000	1.0000	0.8000
750	0.3000	0.4000	0.4000	0.4000
900	0.3000	0.3000	0.2000	0.3000
1050	0.0000	0.0000	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	0.9250	0.9250	1.2951	1.1071	1.4120	11.347	4	14.00	10.00	3	40
*750	0.3750	0.3750	0.6584	0.5796	0.6847	7.979	4	10.00	10.00	25	40
*900	0.2750	0.2750	0.5506	0.4636	0.5796	10.532	4	10.00	10.00	29	40
*1050	0.0500	0.0500	0.2403	0.1588	0.3218	39.161	4	10.00	10.00	38	40

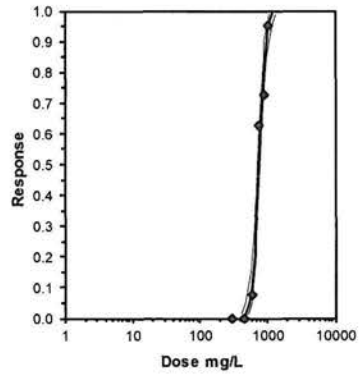
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.89047	0.896	-0.701	1.96258
Equality of variance cannot be confirmed				

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

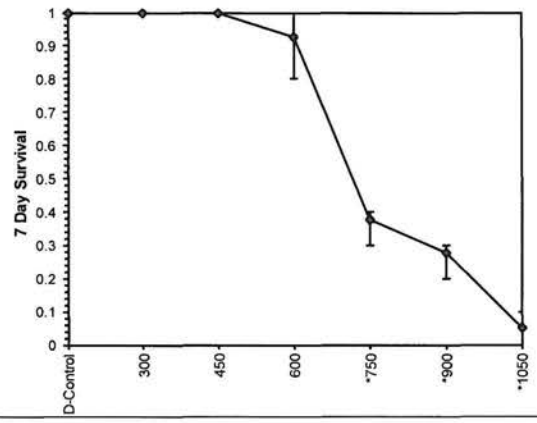
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.5889	1.39472	8.85524	14.3225	0	6.05431	9.48773	0.19513	2.87995	0.08629	3
Intercept	-28.375	4.03268	-36.279	-20.471							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	477.762	407.535	528.936
EC05	3.355	547.038	485.029	592.027
EC10	3.718	587.986	531.674	629.3
EC15	3.964	617.331	565.272	656.205
EC20	4.158	641.694	593.144	678.795
EC25	4.326	663.361	617.809	699.178
EC40	4.747	721.257	682.296	755.842
EC50	5.000	758.493	721.868	794.777
EC60	5.253	797.65	761.304	838.385
EC75	5.674	867.267	826.085	922.443
EC80	5.842	896.55	851.735	959.848
EC85	6.036	931.933	881.829	1006.29
EC90	6.282	978.444	920.256	1069.04
EC95	6.645	1051.68	978.924	1170.97
EC99	7.326	1204.18	1096.47	1392.64



Dose-Response Plot



ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	1/7/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	1/14/2025	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	0.8310	0.6520	0.7570	0.8640
300	0.9030	0.8770	0.9260	0.8370
450	0.7940	0.6790	0.8350	0.8070
600	0.7160	0.6550	0.7190	0.6760
750	0.2200	0.3290	0.2850	0.3260
900	0.2260	0.2200	0.1860	0.1990
1050	0.0000	0.0000	0.0210	0.0530

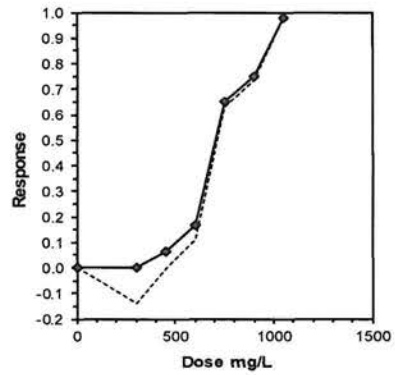
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7760	1.0000	0.7760	0.6520	0.8640	12.113	4				0.8309	1.0000
300	0.8858	1.1414	0.8858	0.8370	0.9260	4.309	4	-2.455	2.290	0.1024	0.8309	1.0000
450	0.7788	1.0035	0.7788	0.6790	0.8350	8.817	4	-0.062	2.290	0.1024	0.7788	0.9373
600	0.6915	0.8911	0.6915	0.6550	0.7190	4.519	4	1.890	2.290	0.1024	0.6915	0.8323
750	0.2900	0.3737	0.2900	0.2200	0.3290	17.517	4				0.2900	0.3490
900	0.2078	0.2677	0.2078	0.1860	0.2260	8.931	4				0.2078	0.2500
1050	0.0185	0.0238	0.0185	0.0000	0.0530	135.351	4				0.0185	0.0223

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94366	0.844	-0.8082	0.44966
Bartlett's Test indicates equal variances (p = 0.28)	3.84372	11.3449		

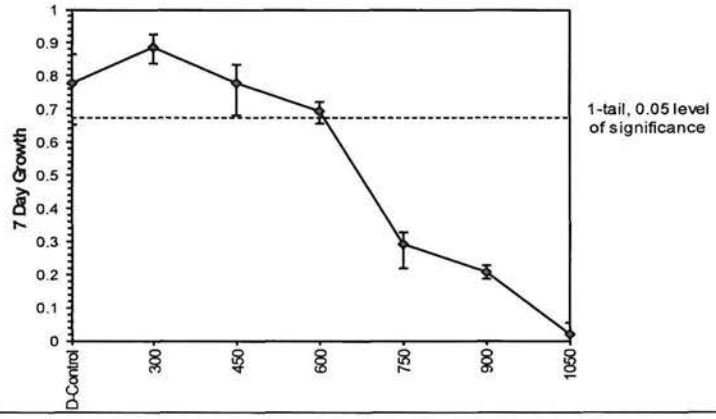
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.10236	0.13191	0.02533	0.004	0.00804	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	419.55	47.38	314.96	558.21	0.0527
IC10	503.23	44.16	351.45	624.34	-0.3623
IC15	574.65	35.08	432.72	634.29	-1.4259
IC20	610.01	16.28	546.69	635.05	-5.2930
IC25	625.53	7.44	600.76	649.53	0.0456
IC40	672.10	6.42	649.86	690.66	0.1055
IC50	703.14	6.72	681.04	723.19	0.0590



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **123**

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.

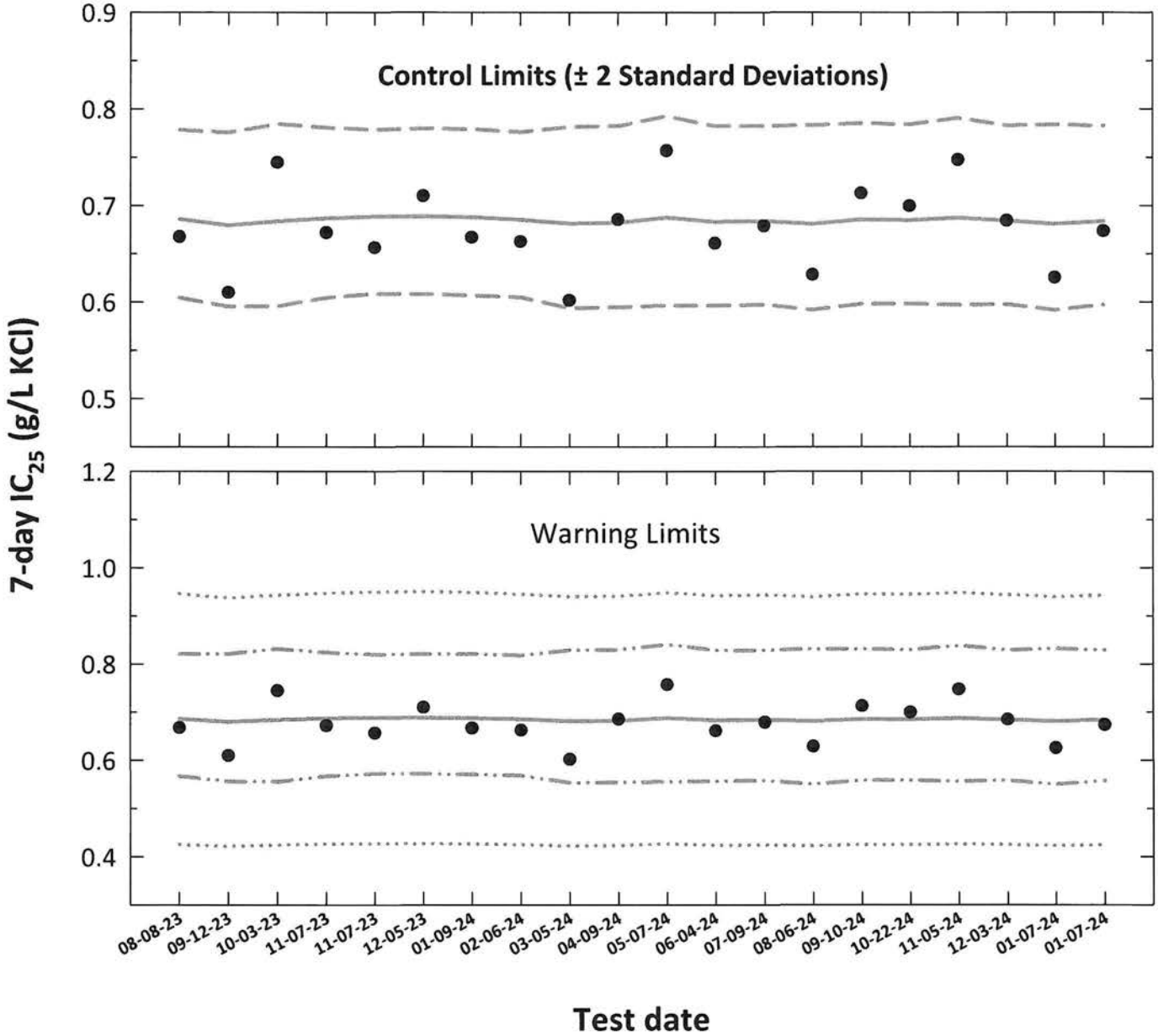
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	EM	EM	EM	EM	EM	EM
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.00	7.92	8.02	7.86	8.01	7.69
	Dissolved oxygen (mg/L)	7.6	7.7	7.8	7.7	8.0	7.5
	Conductivity (µmhos/cm)	316		308		312	
	Alkalinity (mg $CaCO_3$/L)	60		10.15.15		58	
	Hardness (mg $CaCO_3$/L)	87				89	
	Temperature (°C)	24.9	25.0	24.9	25.2	24.8	25.1
300 mg KCl/L	pH (S.U.)	8.03	7.91	8.04	7.83	8.06	7.64
	Dissolved oxygen (mg/L)	7.6	7.7	7.8	7.7	7.7	7.5
	Conductivity (µmhos/cm)	864		838		857	
	Temperature (°C)	24.8	25.2	24.8	24.8	24.9	24.7
450 mg KCl/L	pH (S.U.)	8.03	7.91	8.03	7.82	8.05	7.71
	Dissolved oxygen (mg/L)	7.6	7.7	7.8	7.7	7.7	7.4
	Conductivity (µmhos/cm)	1120		1090		1120	
	Temperature (°C)	24.8	25.2	24.9	24.8	24.9	24.9
600 mg KCl/L	pH (S.U.)	8.04	7.88	8.03	7.81	8.05	7.64
	Dissolved oxygen (mg/L)	7.7	7.7	7.8	7.7	7.7	7.4
	Conductivity (µmhos/cm)	1400		1350		1370	
	Temperature (°C)	24.8	25.0	24.9	24.9	25.0	24.9
750 mg KCl/L	pH (S.U.)	8.04	7.87	8.02	7.81	8.04	7.65
	Dissolved oxygen (mg/L)	7.7	7.7	7.8	7.7	7.7	7.4
	Conductivity (µmhos/cm)	1660		1610		1620	
	Temperature (°C)	24.9	24.9	25.0	25.1	24.8	25.0
900 mg KCl/L	pH (S.U.)	8.05	7.85	8.02	7.84	8.04	7.65
	Dissolved oxygen (mg/L)	7.7	7.6	7.8	7.6	7.7	7.4
	Conductivity (µmhos/cm)	1920		1870		1920	
	Temperature (°C)	24.9	24.9	25.0	24.8	24.8	24.8
1050 mg KCl/L	pH (S.U.)	8.06	7.91	8.02	7.84	8.04	7.75
	Dissolved oxygen (mg/L)	7.7	7.6	7.9	7.6	7.8	7.5
	Conductivity (µmhos/cm)	2210		2130		2160	
	Temperature (°C)	25.0	24.9	24.9	24.8	24.8	25.0
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **123**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	Em	K	K	K	K	Em	Em	Em
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	8.03	7.70	8.20	7.69	8.21	7.74	7.93	7.79
	Dissolved oxygen (mg/L)	7.7	7.0	7.7	6.9	8.0	7.1	8.0	7.4
	Conductivity (µmhos/cm)	305		291		307		312	
	Alkalinity (mg $CaCO_3$/L)			59					
	Hardness (mg $CaCO_3$/L)	K 01-15-25		81				K 01-15-25	
	Temperature (°C)	24.8	25.1	24.7	24.9	24.8	24.7	24.7	25.0
300 mg KCl/L	pH (S.U.)	8.03	7.64	8.11	7.61	8.10	7.72	8.01	7.75
	Dissolved oxygen (mg/L)	7.7	7.3	7.6	7.0	7.7	7.1	7.9	7.3
	Conductivity (µmhos/cm)	852		813		826		848	
	Temperature (°C)	24.9	24.8	24.7	24.9	24.9	24.9	24.7	24.8
450 mg KCl/L	pH (S.U.)	8.03	7.65	8.09	7.64	8.09	7.76	8.01	7.79
	Dissolved oxygen (mg/L)	7.8	7.6	7.7	7.0	7.8	7.1	7.9	7.2
	Conductivity (µmhos/cm)	1100		1070		1070		1110	
	Temperature (°C)	24.9	24.8	24.7	24.7	24.8	24.9	24.8	24.7
600 mg KCl/L	pH (S.U.)	8.03	7.69	8.09	7.58	8.09	7.74	8.00	7.76
	Dissolved oxygen (mg/L)	7.8	9.0	7.8	7.0	7.9	7.0	7.9	7.4
	Conductivity (µmhos/cm)	1370 ~~1370~~ Em 01-10-25		1336		1330		1370	
	Temperature (°C)	25.0	24.7	24.8	24.8	24.8	24.6	24.7	25.1
750 mg KCl/L	pH (S.U.)	8.03	7.63	8.08	7.55	8.09	7.72	8.00	7.86
	Dissolved oxygen (mg/L)	7.8	7.0	7.8	6.9	7.9	7.0	8.0	7.4
	Conductivity (µmhos/cm)	1640		1570		1580		1630	
	Temperature (°C)	24.9	24.9	24.7	24.9	24.8	24.8	24.7	24.7
900 mg KCl/L	pH (S.U.)	8.03	7.65	8.09	7.62	8.09	7.77	8.00	7.84
	Dissolved oxygen (mg/L)	7.8	6.9	7.8	6.9	7.9	7.0	8.0	7.4
	Conductivity (µmhos/cm)	1890		1850		1820		1890	
	Temperature (°C)	24.9	24.6	24.8	25.1	25.0	24.8	24.7	24.7
1050 mg KCl/L	pH (S.U.)	8.03	7.68	8.09	7.61	8.09	7.77	8.00	7.79
	Dissolved oxygen (mg/L)	7.8	7.0	7.8	6.8	7.9	7.0	8.0	7.3
	Conductivity (µmhos/cm)	2160		2100		2100		2170	
	Temperature (°C)	25.0	24.6	24.8	24.9	24.8	24.8	24.7	25.0
		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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	01-07-24	**0.6255**	-0.2038	-0.1668	0.0305	0.6811	0.5917	0.7840	0.5499	0.8321	0.4223	0.9399
20	01-07-24	**0.6737**	-0.1715	-0.1652	0.0293	0.6836	0.5973	0.7825	0.5573	0.8282	0.4239	0.9434

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.

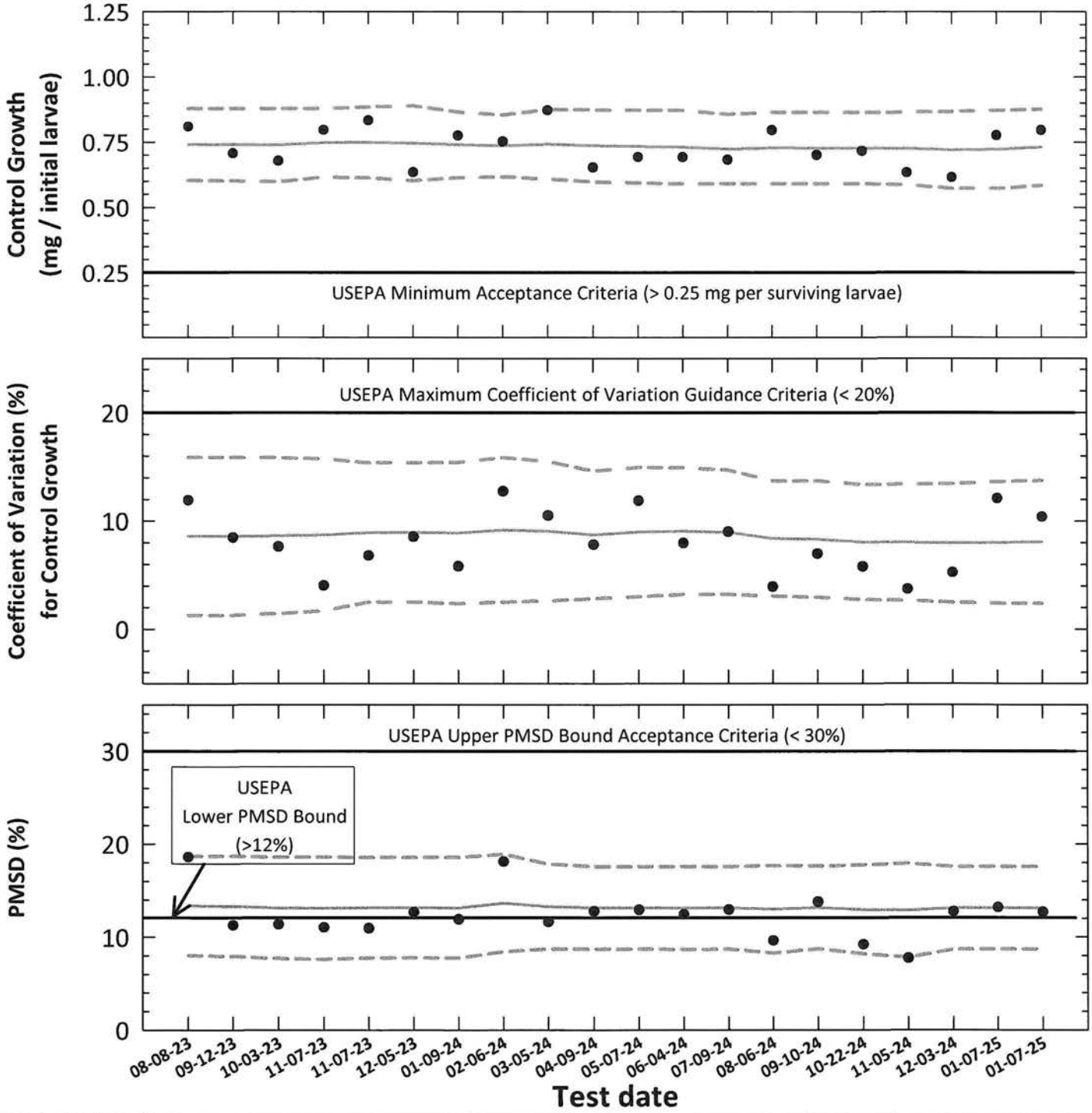
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

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)

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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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	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
19	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	13.1	8.7	17.6
20	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	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
JS

Environmental Testing Solutions, Inc.

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

Species: *Pimephales promelas*

PpKClCR Test Number: **124 - New Artemia**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 7348				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	BLUE
Age:	< 24-hours old	Incubator number and shelf location:	7C
Spawn date:	12-31-24	*Artemia* CHM number:	CHM 1385
Hatch dates and times:	01-06-25 1425 TO 01-07-25 0500	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.37 S.U. Temperature = 24.0 °C	Date / Time in oven:	01-14-25 0600
		*Initial oven temperature:	60 °C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	01-15-25 0600
		*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	01-07-25	0505	JK	1430	JK	0711	JK	01-02-25 B
1	01-08-25	0500	JK	1100	JK	0710	JK	↓
2	01-09-25	0500	JK	1215	JK	0710	JK	01-06-25
3	01-10-25	0500	JK	1100	JK	0700	JK	↓
4	01-11-25	0600	JK	1300	JK	0800	JK	01-07-25
5	01-12-25	0600	JK	1200	JK	0800	JK	↓
6	01-13-25	0500	JK	1230	JK	0735	JK	↓
7	01-14-25					0526	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)	770.8
Average weight per initial larvae:	0.796		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.796	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	673.7

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas***

PpKClCR Test Number: **124 - New Artemia**

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: Magenta Analyst: JP Date: 01-02-24	12.19	14.00	14.11	13.61	14.34	14.89	12.44	13.19	11.98	13.57	15.49	14.97
*B = Pan + Larvae weight (mg) Analyst: Em Date: 01-20-25	19.85	21.21	21.95	22.75	23.60	23.44	21.89	22.55	20.17	22.36	24.22	22.81
C = Larvae weight (mg) = B – A Analyst: JI	7.66	7.21	7.84	9.14	9.26	8.55	9.45	9.36	8.19	8.79	8.73	7.84
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JI	0.766	0.721	0.784	0.914	0.926	0.855	0.945	0.936	0.819	0.879	0.873	0.784
Average weight per initial number of larvae (mg)	0.796				0.916				0.839			
Percent reduction from control (%)							-15.0%				-5.3%	

*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: **124 - New Artemia**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	9 1d	10	10	10
2	10	10	10	10	8 2d	8 2d	9 1d	8 2d	4 5d	4 6d	4 4d	6 4d
3	10	9 1d	10	10	8	7 1d	8 1d	8	4	4	4	5 1d
4	10	9	10	10	8	7	8	8	3 1d	2 2d	3 1d	3 2d
5	10	9	10	10	6 2d	6 1d	8	8	2 1d	2	2 1d	2 1d
6	10	9	10	10	6	6	8	8	2	2	2	2
7	9 1d	9	10	10	5 1d	5 1d	6 2d	6 2d	2	2	2	2
*A = Pan weight (mg) Tray color code: magenta Analyst: JP Date: 01-22-25	13.86	14.78	13.99	13.99	15.71	12.79	13.75	13.16	12.58	13.39	14.72	13.47
*B = Pan + Larvae weight (mg) Analyst: EM Date: 01-20-25	21.43	22.97	21.66	23.10	20.48	16.65	18.24	18.61	14.55	15.61	16.10	14.76
C = Larvae weight (mg) = B – A Analyst: JL	7.57	8.19	7.67	9.11	4.77	3.86	4.49	5.45	1.97	2.22	1.38	1.29
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.757	0.819	0.767	0.911	0.477	0.386	0.449	0.545	0.197	0.222	0.138	0.129
Average weight per initial number of larvae (mg)	0.814				0.464				0.172			
Percent reduction from control (%)			-2.2%				41.7%				78.5%	

*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*** PpKCICR Test Number: **124 - New Artemia**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	8 d	8 d	9 d	9 d
2	3 sk	3 sd	2 d	3 bd
3	1 d	2 d	2	2 d
4	1	1 d	1 d	1 d
5	0 d	0 d	0 d	0 d
6	0	0	0	0
7	0	0	0	0
*A = Pan weight (mg) Tray color code: magenta Analyst: JP Date: 01-02-25	12.99	13.35	13.77	13.88
*B = Pan + Larvae weight (mg) Analyst: ____ Date: ____				
C = Larvae weight (mg) = B – A Analyst: ____				JL 01.12.25
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0	0	0	0
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0		100%	

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

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

Comments:

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

Test type:	***P. promelas* Chronic Reference Toxicant Test**
Test dates:	**January 07-14, 2025**
Associated test:	**PpKClCR # 124 - New Artemia**

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

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

	1st Weight	2nd Weight	Difference (mg)	Percent Difference from 1st Weight (%)
Analyst:		XL		
Tray color code:		Magenta		
Date:		01-23-25		
A		19.79		
B		21.16		
C		21.88		
D		22.68		
E		23.55		
F		23.39		
G		21.83		
H		22.48		
I		20.11		
J		22.28		
K		24.15		
L		22.74		
M		21.38		
N		22.91		
O		21.60		
P		23.04		
Q		20.43		
R		16.60		
S		18.16		
T		18.56		
U		14.50		
V		15.57		
W		16.06		
X		14.73		
Y		-----		
Z		-----		
AA		-----		
BB		-----		
		Average		

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

Test type:	***P. promelas* Chronic Reference Toxicant Test**
Test dates:	**January 07-14, 2025**
Associated test:	**PpKClCR # 124**

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

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

	1st Weight	2nd Weight		
Analyst:	EM	XL	Difference (mg)	Percent Difference from 1st Weight (%)
Tray color code:	Forest Green	Forest Green		
Date:	01-20-25	01-23-25		
A	19.85	19.79	-0.06	-0.30
B	21.21	21.16	-0.05	-0.24
C	21.95	21.88	-0.07	-0.32
D	22.75	22.68	-0.07	-0.31
E	23.60	23.55	-0.05	-0.21
F	23.44	23.39	-0.05	-0.21
G	21.89	21.83	-0.06	-0.27
H	22.55	22.48	-0.07	-0.31
I	20.17	20.11	-0.06	-0.30
J	22.36	22.28	-0.08	-0.36
K	24.22	24.15	-0.07	-0.29
L	22.81	22.74	-0.07	-0.31
M	21.43	21.38	-0.05	-0.23
N	22.97	22.91	-0.06	-0.26
O	21.66	21.60	-0.06	-0.28
P	23.10	23.04	-0.06	-0.26
Q	20.48	20.43	-0.05	-0.24
R	16.65	16.60	-0.05	-0.30
S	18.24	18.16	-0.08	-0.44
T	18.61	18.56	-0.05	-0.27
U	14.55	14.50	-0.05	-0.34
V	15.61	15.57	-0.04	-0.26
W	16.10	16.06	-0.04	-0.25
X	14.76	14.73	-0.03	-0.20
Y	0.00	0.00		
Z	0.00	0.00		
AA	0.00	0.00		
BB	0.00	0.00		
		Average	-0.06	-0.28

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: 124 - New Artemia

Test dates: January 07-14, 2025

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	12.19	19.85	7.66	0.766	0.796	10.4	0.766	100.0	0.796	10.4	Not applicable
	B	10	10	14.00	21.21	7.21	0.721			0.721				
	C	10	10	14.11	21.95	7.84	0.784			0.784				
	D	10	10	13.61	22.75	9.14	0.914			0.914				
300	E	10	10	14.34	23.60	9.26	0.926	0.916	4.5	0.926	100.0	0.916	4.5	-15.0
	F	10	10	14.89	23.44	8.55	0.855			0.855				
	G	10	10	12.44	21.89	9.45	0.945			0.945				
	H	10	10	13.19	22.55	9.36	0.936			0.936				
450	I	10	10	11.98	20.17	8.19	0.819	0.839	5.4	0.819	100.0	0.839	5.4	-5.3
	J	10	10	13.57	22.36	8.79	0.879			0.879				
	K	10	10	15.49	24.22	8.73	0.873			0.873				
	L	10	10	14.97	22.81	7.84	0.784			0.784				
600	M	10	9	13.86	21.43	7.57	0.841	0.857	8.0	0.757	95.0	0.814	8.7	-2.2
	N	10	9	14.78	22.97	8.19	0.910			0.819				
	O	10	10	13.99	21.66	7.67	0.767			0.767				
	P	10	10	13.99	23.10	9.11	0.911			0.911				
750	Q	10	5	15.71	20.48	4.77	0.954	0.846	11.9	0.477	55.0	0.464	14.2	41.7
	R	10	5	12.79	16.65	3.86	0.772			0.386				
	S	10	6	13.75	18.24	4.49	0.748			0.449				
	T	10	6	13.16	18.61	5.45	0.908			0.545				
900	U	10	2	12.58	14.55	1.97	0.985	0.858	26.4	0.197	20.0	0.172	26.4	78.5
	V	10	2	13.39	15.61	2.22	1.110			0.222				
	W	10	2	14.72	16.10	1.38	0.690			0.138				
	X	10	2	13.47	14.76	1.29	0.645			0.129				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.000	0.0	0.000	0.0	0.000	0.0	100.0
	Z	10	0	0.00	0.00	0.00	0.000			0.000				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
	BB	10	0	0.00	0.00	0.00	0.000			0.000				

Dunnett's MSD value: 0.1010

PMSD: 12.7

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 JH

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	1/7/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	1/14/2025	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	1.0000	1.0000
750	0.5000	0.5000	0.6000	0.6000
900	0.2000	0.2000	0.2000	0.2000
1050	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.5500	0.5500	0.8357	0.7854	0.8861	6.955	4	10.00	10.00	18	40
*900	0.2000	0.2000	0.4636	0.4636	0.4636	0.000	4	10.00	10.00	32	40
1050	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4			40	40

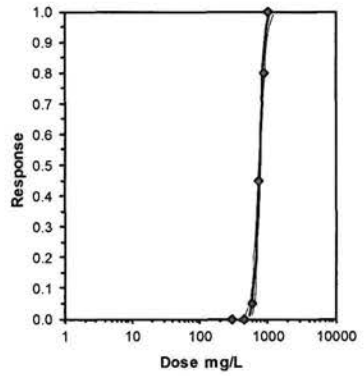
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.79704	0.884	0	1.04658
Equality of variance cannot be confirmed				

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

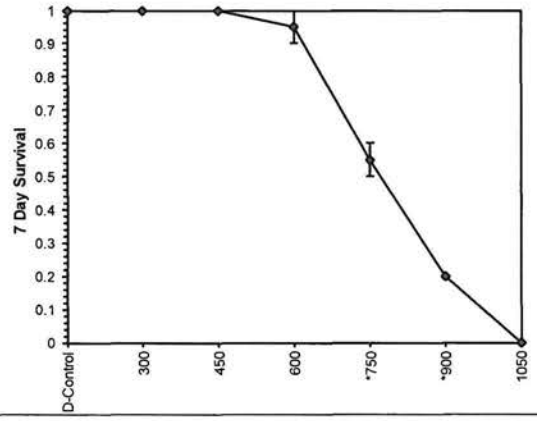
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.2908	1.91841	11.5307	19.0509	0	1.63297	9.48773	0.80286	2.88693	0.0654	4
Intercept	-39.144	5.55793	-50.037	-28.25							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	542.989	477.06	589.139
EC05	3.355	601.672	545.11	641.477
EC10	3.718	635.506	584.786	671.81
EC15	3.964	659.401	612.834	693.465
EC20	4.158	679.032	635.79	711.5
EC25	4.326	696.338	655.883	727.662
EC40	4.747	741.929	707.543	772.026
EC50	5.000	770.781	738.717	802.005
EC60	5.253	800.755	769.454	835.108
EC75	5.674	853.182	819.213	897.755
EC80	5.842	874.928	838.63	925.234
EC85	6.036	900.975	861.191	959.055
EC90	6.282	934.852	889.663	1004.25
EC95	6.645	987.421	932.455	1076.51
EC99	7.326	1094.14	1016.07	1229.13



Dose-Response Plot



Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	1/7/2025	Test ID:	PpKClCR	Sample ID:	REF-Ref Toxicant
End Date:	1/14/2025	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	0.7660	0.7210	0.7840	0.9140
300	0.9260	0.8550	0.9450	0.9360
450	0.8190	0.8790	0.8730	0.7840
600	0.7570	0.8190	0.7670	0.9110
750	0.4770	0.3860	0.4490	0.5450
900	0.1970	0.2220	0.1380	0.1290
1050	0.0000	0.0000	0.0000	0.0000

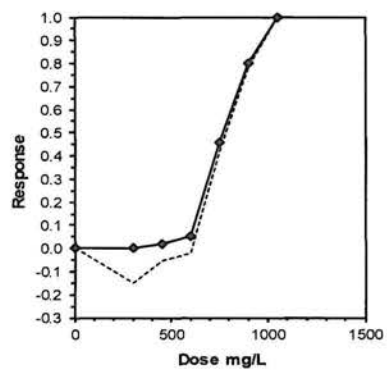
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7963	1.0000	0.7963	0.7210	0.9140	10.405	4				0.8559	1.0000
300	0.9155	1.1498	0.9155	0.8550	0.9450	4.486	4	-2.703	2.290	0.1010	0.8559	1.0000
450	0.8388	1.0534	0.8388	0.7840	0.8790	5.412	4	-0.963	2.290	0.1010	0.8388	0.9800
600	0.8135	1.0217	0.8135	0.7570	0.9110	8.661	4	-0.391	2.290	0.1010	0.8135	0.9505
750	0.4643	0.5830	0.4643	0.3860	0.5450	14.201	4				0.4643	0.5424
900	0.1715	0.2154	0.1715	0.1290	0.2220	26.355	4				0.1715	0.2004
1050	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93887	0.844	0.68832	-0.0341
Bartlett's Test indicates equal variances (p = 0.63)	1.74718	11.3449		

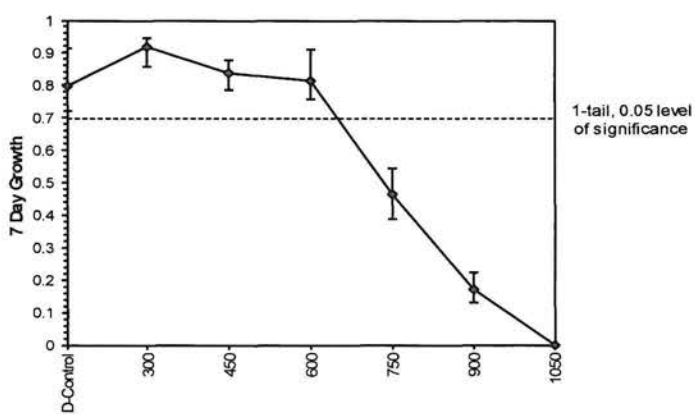
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	600	>600			0.10104	0.1269	0.01109	0.00389	0.08217	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	600.18	70.45	287.82	627.53	-0.8048
IC10	618.56	20.28	518.48	644.25	-1.8400
IC15	636.94	11.51	598.21	663.19	-0.3673
IC20	655.32	10.53	621.08	680.89	-0.2366
IC25	673.70	10.03	642.38	699.64	-0.0376
IC40	728.84	11.55	698.25	764.20	0.2997
IC50	768.61	13.41	724.26	801.37	-0.2361



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **124 - New Artemia**

Daily Chemistry:

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

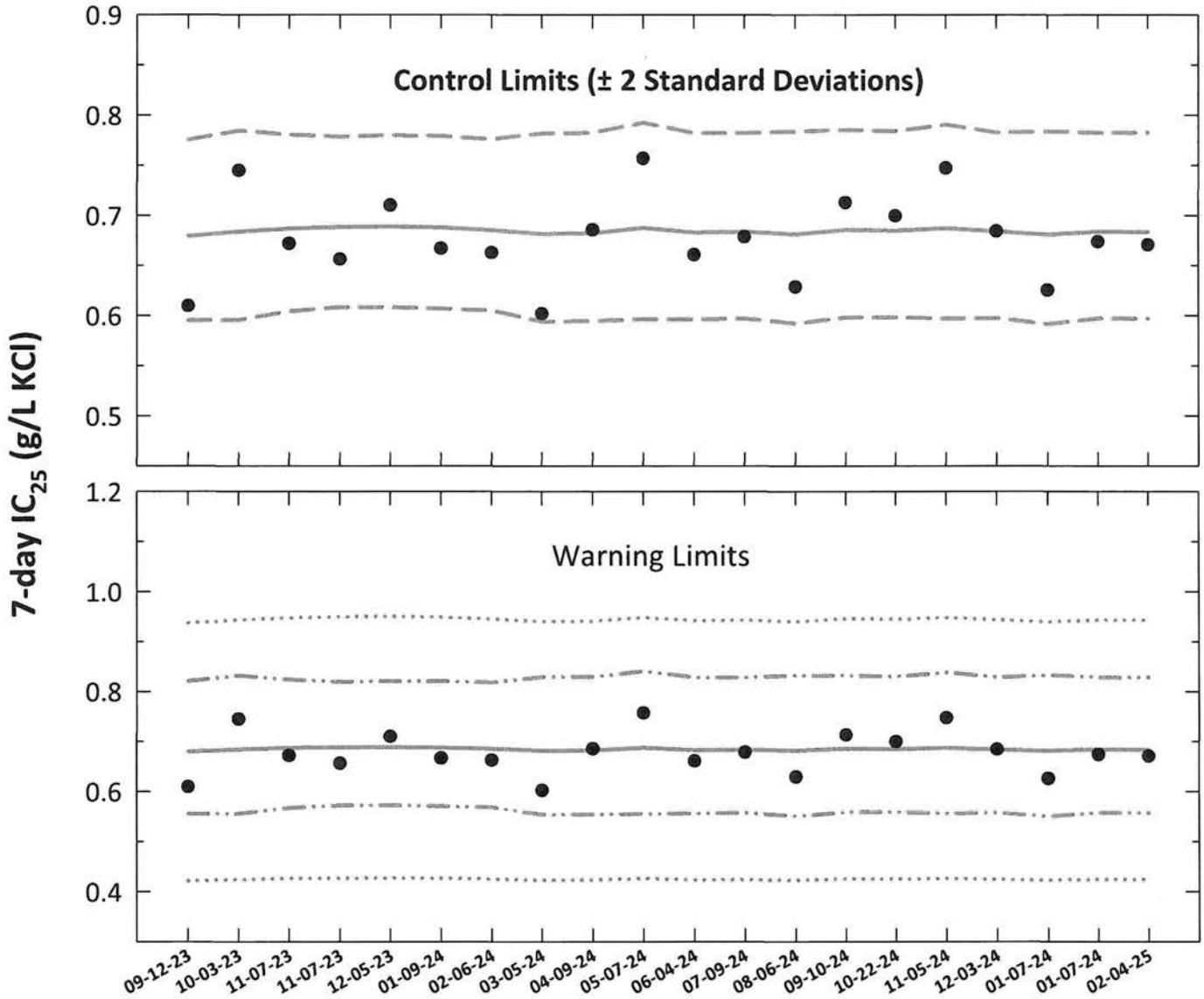
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	EM	EM	EM	EM	EM	EM
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.00	7.41	8.02	7.85	8.01	7.77
	Dissolved oxygen (mg/L)	7.6	7.6	7.8	7.7	8.0	7.4
	Conductivity (µmhos/cm)	316		308		312	
	Alkalinity (mg $CaCO_3$/L)	60				58	
	Hardness (mg $CaCO_3$/L)	87				89	
	Temperature (°C)	24.8	25.0	24.9	25.2	24.8	25.1
300 mg KCl/L	pH (S.U.)	8.03	7.41	8.04	7.85	8.06	7.75
	Dissolved oxygen (mg/L)	7.6	7.5	7.8	7.7	7.7	7.4
	Conductivity (µmhos/cm)	864		838		857	
	Temperature (°C)	24.8	24.9	24.9	25.0	24.9	24.8
450 mg KCl/L	pH (S.U.)	8.03	7.88	8.03	7.84	8.05	7.76
	Dissolved oxygen (mg/L)	7.6	7.6	7.8	7.7	7.7	7.5
	Conductivity (µmhos/cm)	1120		1090		1120	
	Temperature (°C)	24.8	24.9	25.0	24.9	24.9	25.2
600 mg KCl/L	pH (S.U.)	8.04	7.90	8.03	7.83	8.05	7.75
	Dissolved oxygen (mg/L)	7.7	7.6	7.8	7.7	7.7	7.5
	Conductivity (µmhos/cm)	1400		1350		1370	
	Temperature (°C)	24.8	25.1	25.0	24.9	25.0	25.0
750 mg KCl/L	pH (S.U.)	8.04	7.89	8.02	7.83	8.04	7.72
	Dissolved oxygen (mg/L)	7.7	7.6	7.8	7.6	7.7	7.5
	Conductivity (µmhos/cm)	1660		1610		1620	
	Temperature (°C)	25.0	24.8	24.8	24.9	25.0	25.0
900 mg KCl/L	pH (S.U.)	8.05	7.88	8.02	7.80	8.04	7.74
	Dissolved oxygen (mg/L)	7.7	7.7	7.8	7.6	7.7	7.6
	Conductivity (µmhos/cm)	1920		1870		1920	
	Temperature (°C)	25.0	24.8	24.8	25.1	24.9	24.8
1050 mg KCl/L	pH (S.U.)	8.06	7.90	8.02	7.82	8.04	7.78
	Dissolved oxygen (mg/L)	7.7	7.7	7.9	7.7	7.8	7.6
	Conductivity (µmhos/cm)	2210		2130		2160	
	Temperature (°C)	24.9	25.0	24.9	24.8	24.9	24.8
		Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **124 - New Artemia**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	Em	K	K	K	K	Em	Em	Em
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	8.03	7.71	8.20	7.74	8.21	7.70	7.93	7.77
	Dissolved oxygen (mg/L)	7.7	6.4	7.7	6.8	8.0	7.2	8.0	7.2
	Conductivity (µmhos/cm)	305		291		307		312	
	Alkalinity (mg $CaCO_3$/L)			59					
	Hardness (mg $CaCO_3$/L)		K 01-15-25	81					K 01-15-25
	Temperature (°C)	25.0	25.1	24.8	25.1	24.8	24.9	24.8	24.9
300 mg KCl/L	pH (S.U.)	8.03	7.60	8.4	7.50	8.10	7.72	8.01	7.81
	Dissolved oxygen (mg/L)	7.7	6.8	7.6	6.8	7.7	7.1	7.9	7.3
	Conductivity (µmhos/cm)	852		813		826		848	
	Temperature (°C)	24.8	24.8	24.9	24.7	24.9	25.0	24.9	25.1
450 mg KCl/L	pH (S.U.)	8.03	7.65	8.09	7.65	8.09	7.76	8.01	7.78
	Dissolved oxygen (mg/L)	7.8	6.8	7.7	6.9	7.8	7.0	7.9	7.3
	Conductivity (µmhos/cm)	1100		1070		1070		1110	
	Temperature (°C)	24.8	24.8	24.9	24.7	24.9	24.8	24.9	25.1
600 mg KCl/L	pH (S.U.)	8.03	7.70	8.09	7.63	8.09	7.73	8.00	7.75
	Dissolved oxygen (mg/L)	7.8	6.7	7.8	6.9	7.9	7.0	7.9	7.3
	Conductivity (µmhos/cm)	1370		1330		1330		1370	
	Temperature (°C)	24.8	25.0	24.9	24.9	24.8	24.8	25.0	25.0
750 mg KCl/L	pH (S.U.)	8.03	7.68	8.08	7.59	8.09	7.72	8.00	7.77
	Dissolved oxygen (mg/L)	7.8	6.7	7.8	6.9	7.9	7.0	8.0	7.4
	Conductivity (µmhos/cm)	1640		1570		1580		1630	
	Temperature (°C)	24.9	24.7	25.0	24.6	24.8	24.8	25.0	25.2
900 mg KCl/L	pH (S.U.)	8.03	7.61	8.09	7.62	8.09	7.72	8.00	7.78
	Dissolved oxygen (mg/L)	7.8	6.8	7.8	6.9	7.9	7.1	8.0	7.4
	Conductivity (µmhos/cm)	1890		1850		1820		1890	
	Temperature (°C)	24.9	24.7	25.0	24.9	24.8	24.7	25.0	24.7
1050 mg KCl/L	pH (S.U.)	8.03	7.66	8.09	7.68				
	Dissolved oxygen (mg/L)	7.8	7.0	7.8	6.9				
	Conductivity (µmhos/cm)	2160		2100					
	Temperature (°C)	24.8	24.7	25.0	25.0				01-21 K
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	09-12-23	**0.6098**	-0.2148	-0.1677	0.0287	0.6796	0.5954	0.7757	0.5557	0.8211	0.4214	0.9378
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	01-07-24	**0.6255**	-0.2038	-0.1668	0.0305	0.6811	0.5917	0.7840	0.5499	0.8321	0.4223	0.9399
19	01-07-24	**0.6737**	-0.1715	-0.1652	0.0293	0.6836	0.5973	0.7825	0.5573	0.8282	0.4239	0.9434
20	02-04-25	**0.6706**	-0.1735	-0.1653	0.0293	0.6835	0.5971	0.7824	0.5571	0.8282	0.4238	0.9432

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.

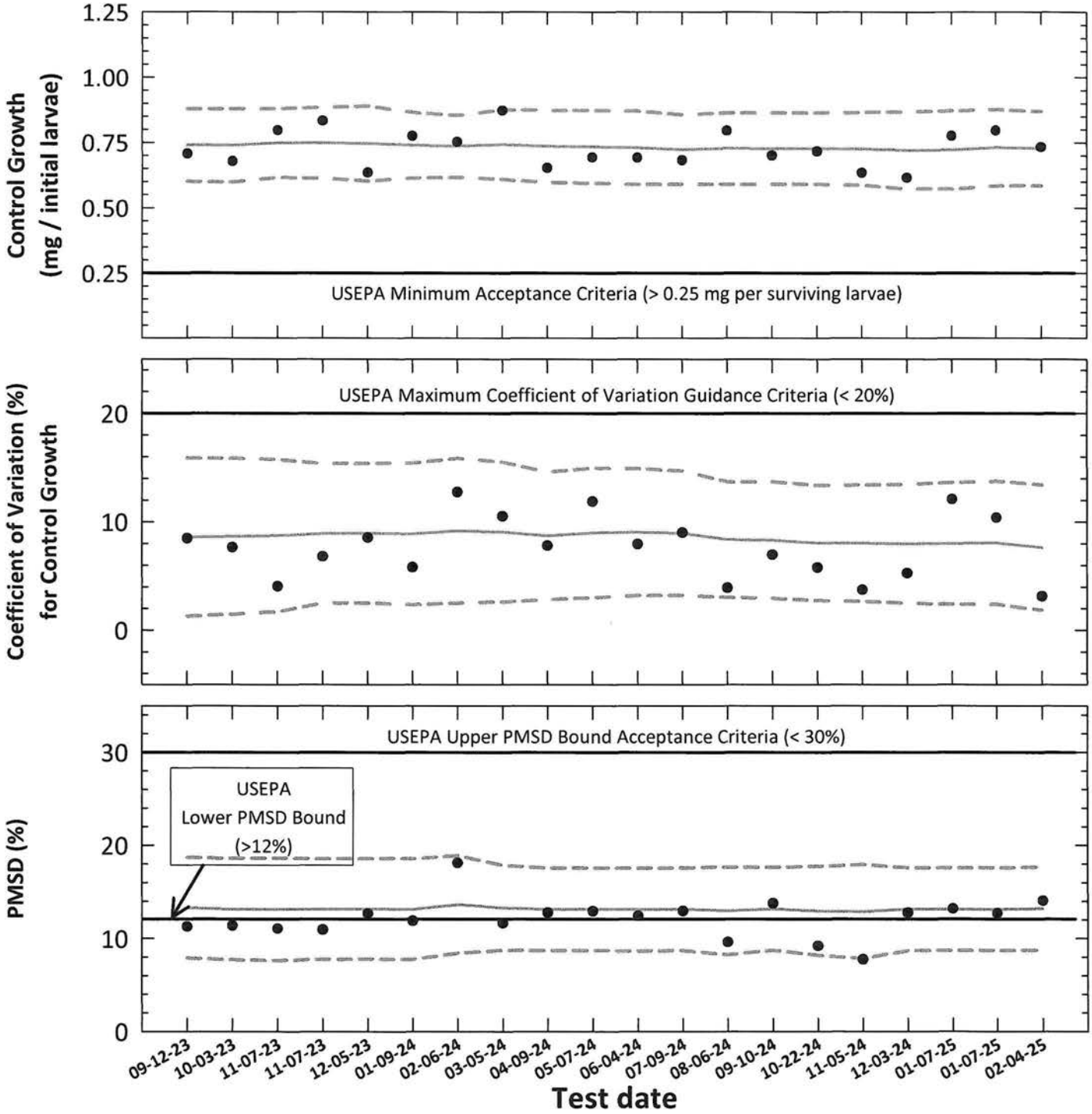
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	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
18	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	13.1	8.7	17.6
19	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	13.1	8.7	17.6
20	02-04-25	100	0.733	3.1	0.1030	14.1	0.727	0.585	0.869	7.6	1.8	13.4	13.2	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: **125**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 7348				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	YELLOW
Age:	< 24-hours old	Incubator number and shelf location:	7 B
Spawn date:	01-28-25	*Artemia* CHM number:	CHM1294
Hatch dates and times:	02-03-25 1440 TO 02-04-25 0450	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.45 S.U. Temperature = 24.0 °C	Date / Time in oven:	02-11-25 0645
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	02-12-25 0645
		*Final oven temperature:	60°C
		Total drying time:	24 HOURS

*60°C Oven, Thermometer SN: 14-985B5

Daily feeding and renewal information:

Day	Date	Morning feeding		Afternoon feeding		Test initiation, renewal, or termination		MHSW batch used
		Time	Analyst	Time	Analyst	Time	Analyst	
0	02-04-25	0500	JK	1430	JK	0711	JK	01-28-25 B
1	02-05-25	0500	JK	1500	JK	0700	JK	↓
2	02-06-25	0500	JK	1300	JK	0700	JK	02-02-25 B
3	02-07-25	0500	JK	1100	JK	0700	JK	↓
4	02-08-25	0500	JK	1200	JK	0821	JK	02-06-25 A
5	02-09-25	0500	JK	1100	JK	0700	JK	↓
6	02-10-25	0500	JK	1200	JK	0700	JK	↓
7	02-11-25					0612	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)	806.0
Average weight per initial larvae:	0.733		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.733	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	670.6

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **125**

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: Lavender Analyst: XL Date: 02-23-25	13.56	14.85	12.25	12.48	12.81	12.57	14.54	14.16	13.15	12.68	14.43	13.64
*B = Pan + Larvae weight (mg) Analyst: XL Date: 02-14-25	21.09	22.23	19.25	19.89	20.23	20.76	24.19	23.05	20.97	20.51	21.33	20.69
C = Larvae weight (mg) = B – A Analyst: JL	7.53	7.38	7.00	7.41	7.42	8.19	9.65	8.89	7.82	7.83	6.90	7.05
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.753	0.738	0.700	0.741	0.742	0.819	0.965	0.889	0.782	0.783	0.690	0.705
Average weight per initial number of larvae (mg)	0.733				0.854				0.740			
Percent reduction from control (%)							-16.5%				-1.0%	

*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: **125**

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	9 1d	10	9 1d	9 1d	8 2d	9 1d	8 2d	4 6d	4 6d	5 5d	5 6d
2	10	9	10	9	9	8	8 1d	8	3 6d	3 1d	5	4 1d
3	9 1d	9	10	9	8 1d	7 1d	7 1d	8	3	3	4 1d	4
4	9	9	10	9	8	7	7	8	3	3	4	4
5	9	9	10	9	8	7	7	8	2 1d	2 1d	3 1d	4
6	9	9	10	9	7 1d	7	7	7 1d	1 1c	2	3	4
7	9	9	10	9	7	7	7	7	1	2	3	3 1d
*A = Pan weight (mg) Tray color code: lavender Analyst: XL Date: 01-23-25	10.77	14.25	13.69	14.26	13.39	13.19	12.18	12.36	14.02	13.59	14.68	10.39
*B = Pan + Larvae weight (mg) Analyst: XL Date: 02-14-25	17.26	20.57	21.18	20.27	18.62	18.95	17.11	17.42	14.71	15.01	16.67	12.17
C = Larvae weight (mg) = B – A Analyst: JL	6.49	6.32	7.49	6.01	5.23	5.76	4.93	5.06	0.69	1.42	1.99	1.78
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.649	0.632	0.749	0.601	0.523	0.576	0.493	0.506	0.069	0.142	0.199	0.178
Average weight per initial number of larvae (mg)	0.658				0.525				0.147			
Percent reduction from control (%)			10.37.				28.47.				79.97.	

*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: **125**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	3 2d	3 2d	3 2d	2 8d
2	1 2d	1 2d	2 1d	1 1d
3	1	1	2	1
4	1	1	2	1
5	0 1d	1	2	1
6	0	1	2	1
7	0	1	2	1
*A = Pan weight (mg) Tray color code: Lavender Analyst: XL Date: 01-23-25	12.27	12.66	14.35	14.73
*B = Pan + Larvae weight (mg) Analyst: XL Date: 02-14-25		13.88	15.65	15.60
C = Larvae weight (mg) = B – A Analyst: JL	02-21-25 JL	1.22	1.30	0.87
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0	0.122	0.130	0.087
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.085		88.4 %	

*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: 125

Test dates: February 04-11, 2025

Concentration (mg/L KCl)	Replicate	Initial number of larvae	Final number of larvae	A = Pan weight (mg)	B = Pan + Larvae weight (mg)	Larvae weight (mg) = B - A	Weight / Surviving number of larvae (mg)	Mean weight/ Surviving number of larvae (mg)	Coefficient of variation (Mean weight per surviving number of larvae) (%)	Weight / Initial number of larvae (mg)	Mean survival (%)	Mean weight/ Initial number of larvae (mg)	Coefficient of variation (%)	Percent reduction from control (%)
Control	A	10	10	13.56	21.09	7.53	0.753	0.733	3.1	0.753	100.0	0.733	3.1	Not applicable
	B	10	10	14.85	22.23	7.38	0.738			0.738				
	C	10	10	12.25	19.25	7.00	0.700			0.700				
	D	10	10	12.48	19.89	7.41	0.741			0.741				
300	E	10	10	12.81	20.23	7.42	0.742	0.854	11.2	0.742	100.0	0.854	11.2	-16.5
	F	10	10	12.57	20.76	8.19	0.819			0.819				
	G	10	10	14.54	24.19	9.65	0.965			0.965				
	H	10	10	14.16	23.05	8.89	0.889			0.889				
450	I	10	10	13.15	20.97	7.82	0.782	0.740	6.7	0.782	100.0	0.740	6.7	-1.0
	J	10	10	12.68	20.51	7.83	0.783			0.783				
	K	10	10	14.43	21.33	6.90	0.690			0.690				
	L	10	10	13.64	20.69	7.05	0.705			0.705				
600	M	10	9	10.77	17.26	6.49	0.721	0.710	4.8	0.649	92.5	0.658	9.7	10.3
	N	10	9	14.25	20.57	6.32	0.702			0.632				
	O	10	10	13.69	21.18	7.49	0.749			0.749				
	P	10	9	14.26	20.27	6.01	0.668			0.601				
750	Q	10	7	13.39	18.62	5.23	0.747	0.749	7.0	0.523	70.0	0.525	7.0	28.4
	R	10	7	13.19	18.95	5.76	0.823			0.576				
	S	10	7	12.18	17.11	4.93	0.704			0.493				
	T	10	7	12.36	17.42	5.06	0.723			0.506				
900	U	10	1	14.02	14.71	0.69	0.690	0.664	7.7	0.069	22.5	0.147	38.8	79.9
	V	10	2	13.59	15.01	1.42	0.710			0.142				
	W	10	3	14.68	16.67	1.99	0.663			0.199				
	X	10	3	10.39	12.17	1.78	0.593			0.178				
1050	Y	10	0	0.00	0.00	0.00	0.000	0.913	31.5	0.000	10.0	0.085	70.2	88.4
	Z	10	1	12.66	13.88	1.22	1.220			0.122				
	AA	10	2	14.35	15.65	1.30	0.650			0.130				
	BB	10	1	14.73	15.60	0.87	0.870			0.087				

Dunnett's MSD value: 0.1030 MSD = Minimum Significant Difference

PMSD: 14.1 PMSD = Percent Minimum Significant Difference

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

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

Prepared and Reviewed by Jim Swinner

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Sumner

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	2/4/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	2/11/2025	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	1.0000	0.9000
750	0.7000	0.7000	0.7000	0.7000
900	0.1000	0.2000	0.3000	0.3000
1050	0.0000	0.1000	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	0.9250	0.9250	1.2898	1.2490	1.4120	6.318	4	12.00	10.00	3	40
*750	0.7000	0.7000	0.9912	0.9912	0.9912	0.000	4	10.00	10.00	12	40
*900	0.2250	0.2250	0.4862	0.3218	0.5796	25.196	4	10.00	10.00	31	40
*1050	0.1000	0.1000	0.3165	0.1588	0.4636	39.374	4	10.00	10.00	36	40

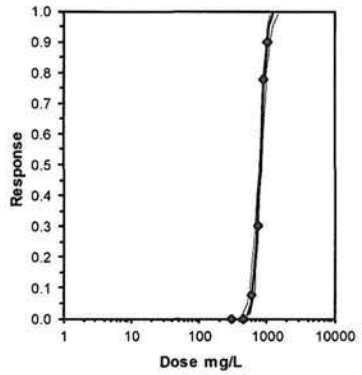
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.77593	0.896	-0.2907	2.60976
Equality of variance cannot be confirmed				

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

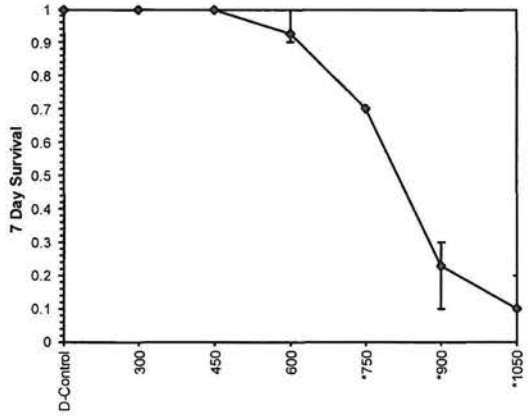
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.106	1.52107	9.12467	15.0873	0	1.46603	9.48773	0.83264	2.90636	0.0826	3
Intercept	-30.184	4.4291	-38.865	-21.503							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	517.84	442.804	571.213
EC05	3.355	589.508	524.266	635.791
EC10	3.718	631.684	573.068	673.839
EC15	3.964	661.832	608.1	701.288
EC20	4.158	686.815	637.071	724.346
EC25	4.326	708.999	662.625	745.178
EC40	4.747	768.128	728.929	803.361
EC50	5.000	806.048	769.135	843.628
EC60	5.253	845.84	808.819	888.913
EC75	5.674	916.382	873.478	976.173
EC80	5.842	945.98	899	1014.88
EC85	6.036	981.69	928.92	1062.81
EC90	6.282	1028.54	967.094	1127.4
EC95	6.645	1102.13	1025.3	1231.94
EC99	7.326	1254.66	1141.56	1458.13



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:	2/4/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	2/11/2025	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	0.7530	0.7380	0.7000	0.7410
300	0.7420	0.8190	0.9650	0.8890
450	0.7820	0.7830	0.6900	0.7050
600	0.6490	0.6320	0.7490	0.6010
750	0.5230	0.5760	0.4930	0.5060
900	0.0690	0.1420	0.1990	0.1780
1050	0.0000	0.1220	0.1300	0.0870

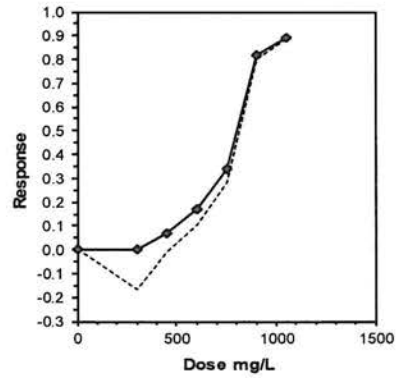
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7330	1.0000	0.7330	0.7000	0.7530	3.129	4				0.7934	1.0000
300	0.8538	1.1647	0.8538	0.7420	0.9650	11.177	4	-2.686	2.290	0.1030	0.7934	1.0000
450	0.7400	1.0095	0.7400	0.6900	0.7830	6.683	4	-0.156	2.290	0.1030	0.7400	0.9327
600	0.6578	0.8973	0.6578	0.6010	0.7490	9.730	4	1.674	2.290	0.1030	0.6578	0.8291
750	0.5245	0.7156	0.5245	0.4930	0.5760	6.952	4				0.5245	0.6611
900	0.1470	0.2005	0.1470	0.0690	0.1990	38.829	4				0.1470	0.1853
1050	0.0848	0.1156	0.0848	0.0000	0.1300	70.213	4				0.0848	0.1068

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97542	0.844	0.20746	0.17594
Bartlett's Test indicates equal variances (p = 0.21)	4.52375	11.3449		

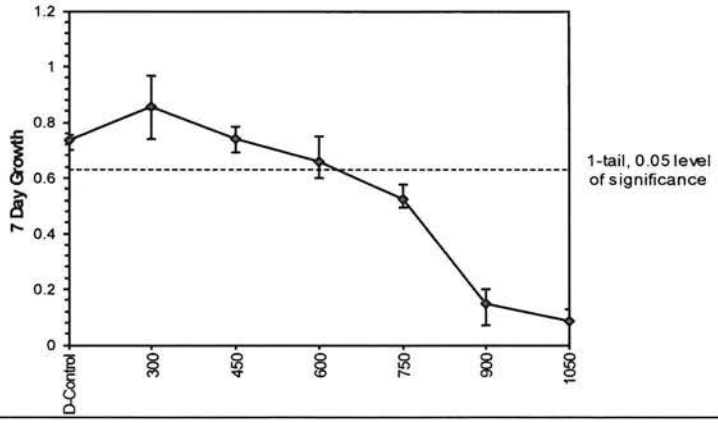
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	600	>600			0.10296	0.14047	0.02614	0.00404	0.0075	3, 12
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	411.48	53.27	316.27	601.01	0.6510
IC10	497.35	56.88	347.91	675.66	0.2778
IC15	569.69	48.65	395.99	688.87	-0.2348
IC20	625.95	35.76	499.17	706.57	-0.4258
IC25	670.60	29.89	552.80	737.05	-0.4291
IC40	769.26	7.60	744.56	792.76	-0.0217
IC50	800.79	6.62	778.41	821.45	-0.0697



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **125**

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.

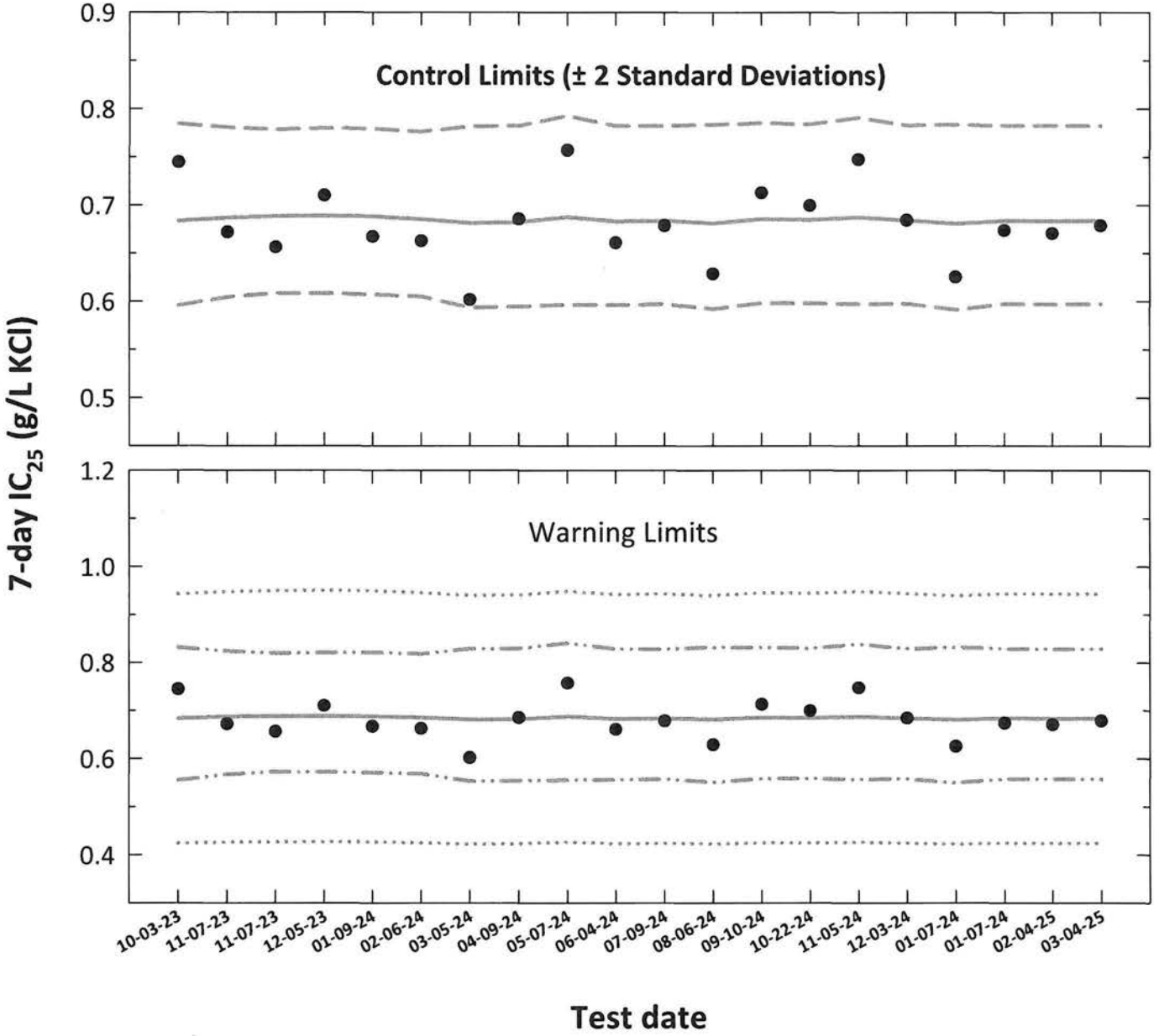
		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)					
		0		1		2	
	Analyst	N	Em	Em	Em	Em	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	8.05	7.80	7.82	7.77	7.91	7.58
	Dissolved oxygen (mg/L)	8.0	7.6	7.9	7.7	7.9	6.5
	Conductivity (µmhos/cm)	300		312		306	
	Alkalinity (mg $CaCO_3$/L)	59			0.12-25 N	60	
	Hardness (mg $CaCO_3$/L)	90				86	
	Temperature (°C)	24.7	25.1	24.8	25.0	24.7	24.9
300 mg KCl/L	pH (S.U.)	8.03	7.77	7.91	7.72	7.94	7.45
	Dissolved oxygen (mg/L)	8.0	7.6	7.8	7.6	7.9	6.4
	Conductivity (µmhos/cm)	844		852		841	
	Temperature (°C)	24.8	25.0	24.9	24.9	24.8	24.5
450 mg KCl/L	pH (S.U.)	8.01	7.76	7.90	7.73	7.94	7.45
	Dissolved oxygen (mg/L)	8.0	7.6	7.9	7.6	7.9	6.4
	Conductivity (µmhos/cm)	1090		1120		1120	
	Temperature (°C)	24.8	25.2	24.8	24.7	24.7	24.5
600 mg KCl/L	pH (S.U.)	8.00	7.78	7.89	7.72	7.93	7.44
	Dissolved oxygen (mg/L)	8.0	7.6	7.9	7.6	8.0	6.5
	Conductivity (µmhos/cm)	1350		1380		1370	
	Temperature (°C)	24.9	25.2	24.8	24.6	24.7	24.8
750 mg KCl/L	pH (S.U.)	7.99	7.72	7.89	7.72	7.93	7.44
	Dissolved oxygen (mg/L)	8.0	7.6	7.9	7.7	7.9	6.3
	Conductivity (µmhos/cm)	1620		1640		1630	
	Temperature (°C)	24.7	24.9	24.7	24.9	24.7	24.9
900 mg KCl/L	pH (S.U.)	7.99	7.76	7.89	7.73	7.93	7.44
	Dissolved oxygen (mg/L)	8.0	7.5	7.8	7.7	8.0	6.3
	Conductivity (µmhos/cm)	1880		1900		1880	
	Temperature (°C)	24.7	24.9	24.7	24.9	24.8	24.9
1050 mg KCl/L	pH (S.U.)	8.00	7.80	7.89	7.79	7.93	7.54
	Dissolved oxygen (mg/L)	7.9	7.5	7.8	7.8	7.9	6.8
	Conductivity (µmhos/cm)	2140		2160		2140	
	Temperature (°C)	24.9	24.9	24.7	24.7	24.6	24.7
		Initial	Final	Initial	Final	Initial	Final

ETS
Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **125**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	N	N	N	N	N	EM	EM	N
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	8.02	7.62	8.10	7.59	8.09	7.84	7.97	7.50
	Dissolved oxygen (mg/L)	7.8	6.4	7.9 ~~293~~ N 020825	6.5	8.0	7.8	8.1	6.0
	Conductivity (µmhos/cm)	300		293		296		310	
	Alkalinity (mg $CaCO_3$/L)		N 02.12.25 N	59					
	Hardness (mg $CaCO_3$/L)			58				N 02.12.25 N	
	Temperature (°C)	24.8	24.9	24.8	24.7	24.7	25.0	24.7	24.7
300 mg KCl/L	pH (S.U.)	7.99	7.45	8.03	7.47	8.05	7.84	8.05	7.34
	Dissolved oxygen (mg/L)	7.8	6.4	7.9	6.4	8.0	7.8	8.0	5.8
	Conductivity (µmhos/cm)	814		806		808		855	
	Temperature (°C)	24.9	24.7	24.9	24.5	24.7	24.6	24.8	24.9
450 mg KCl/L	pH (S.U.)	7.96	7.46	8.03	7.49	8.03	7.78	8.05	7.32
	Dissolved oxygen (mg/L)	7.8	6.3	7.9	6.4	8.0	7.8	8.0	5.3
	Conductivity (µmhos/cm)	1060		1060		1070		1120	
	Temperature (°C)	24.9	24.6	24.9	24.6	24.8	24.7	24.8	24.9
600 mg KCl/L	pH (S.U.)	7.96	7.45	8.02	7.48	8.03	7.79	8.04	7.31
	Dissolved oxygen (mg/L)	7.9	6.2	7.9	6.5	8.0	7.7	8.0	5.3
	Conductivity (µmhos/cm)	1320		1320		1330		1400	
	Temperature (°C)	24.8	24.6	25.0	24.8	24.9	24.6	24.9	24.5
750 mg KCl/L	pH (S.U.)	7.96	7.44	8.04	7.51	8.02	7.77	8.04	7.30
	Dissolved oxygen (mg/L)	7.9	6.2	7.9	6.6	8.0	7.6	8.1	5.3
	Conductivity (µmhos/cm)	1560		1550		1580		1650	
	Temperature (°C)	24.8	24.8	25.0	24.8	25.0	24.9	24.9	24.8
900 mg KCl/L	pH (S.U.)	7.95	7.63	8.03	7.52	8.02	7.87	8.04	7.34
	Dissolved oxygen (mg/L)	7.9	7.0	7.9	6.6	8.0	7.6	8.1	5.5
	Conductivity (µmhos/cm)	1820		1820		1840		1920	
	Temperature (°C)	24.8	24.7	25.0	24.7	25.0	24.9	24.9	24.8
1050 mg KCl/L	pH (S.U.)	7.95	7.53	8.03	7.62	8.02	7.82	8.04	7.35
	Dissolved oxygen (mg/L)	7.9	6.1	7.9	6.4	8.1	7.7	8.1	5.4
	Conductivity (µmhos/cm)	2110		2090		2070		2170	
	Temperature (°C)	24.8	24.5	25.0	24.9	25.0	24.7	25.0	24.9
		Initial	Final	Initial	Final	Initial	Final	Initial	Final

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



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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

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

Test number	Test date	7-day IC_{25} ToxCal Determination (g/L KCl)	Log_{10} Conversion			Anti-logarithmic Values (g/L KCl)						
			7-day IC_{25}	CT	S	CT	Control Limits		Laboratory Calculated CV Warning Limits		75th Percentile CV Warning Limits	
							CT - 2S	CT + 2S	CT - 2CV	CT + 2CV	CT - $S_{A.75}$	CT + $S_{A.75}$
1	10-03-23	0.7447	-0.1280	-0.1651	0.0299	0.6837	0.5959	0.7846	0.5552	0.8312	0.4239	0.9436
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	01-07-24	0.6255	-0.2038	-0.1668	0.0305	0.6811	0.5917	0.7840	0.5499	0.8321	0.4223	0.9399
18	01-07-24	0.6737	-0.1715	-0.1652	0.0293	0.6836	0.5973	0.7825	0.5573	0.8282	0.4239	0.9434
19	02-04-25	0.6706	-0.1735	-0.1653	0.0293	0.6835	0.5971	0.7824	0.5571	0.8282	0.4238	0.9432
20	03-04-25	0.6786	-0.1684	-0.1650	0.0293	0.6839	0.5976	0.7826	0.5577	0.8283	0.4240	0.9438

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.

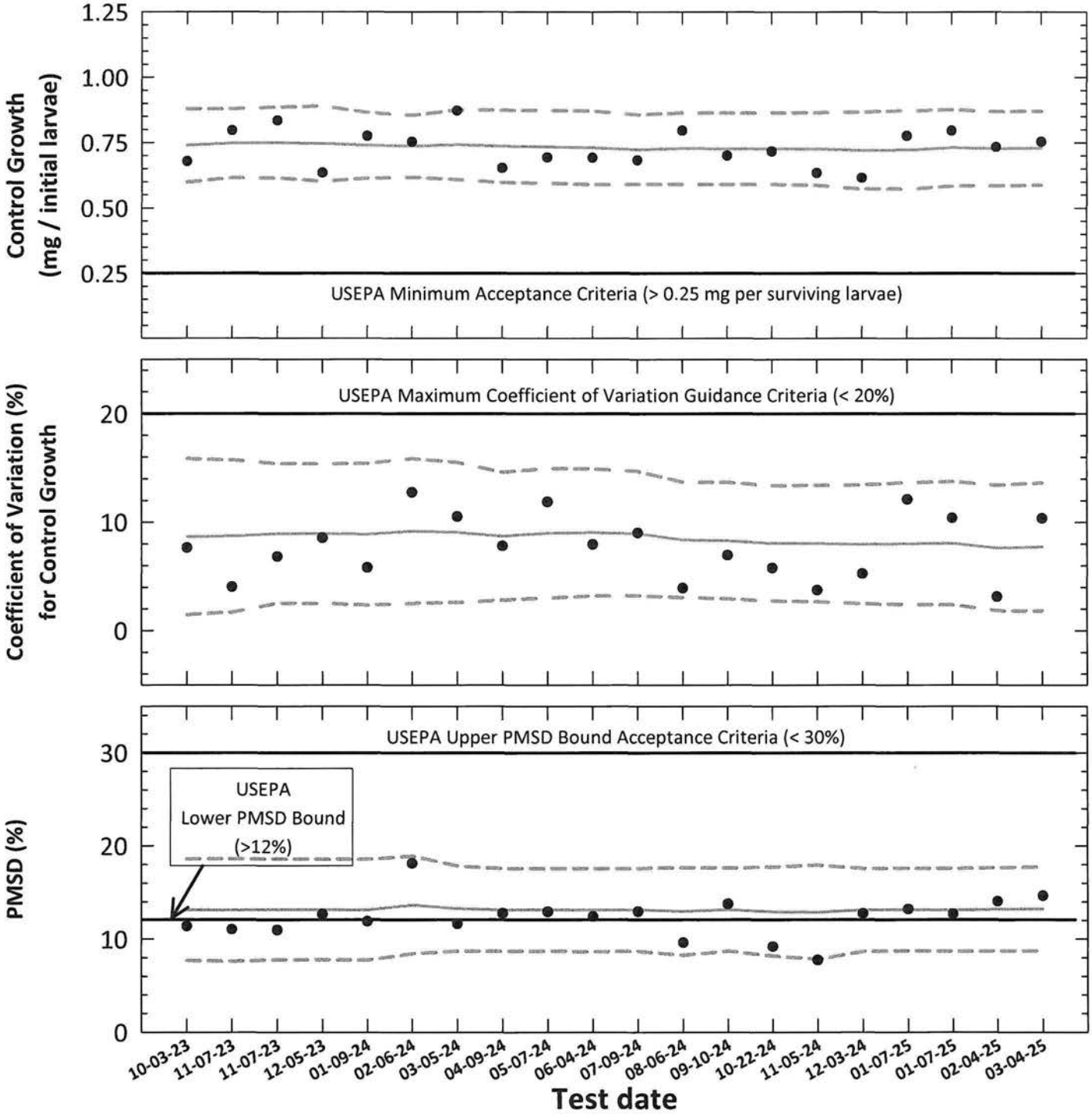
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

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	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13	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
14	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
15	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
16	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
17	01-07-25	100	0.776	12.1	0.1024	13.2	0.723	0.573	0.872	8.0	2.4	13.7	13.1	8.7	17.6
18	01-07-25	100	0.796	10.4	0.1010	12.7	0.731	0.585	0.877	8.1	2.4	13.8	13.1	8.7	17.6
19	02-04-25	100	0.733	3.1	0.1030	14.1	0.727	0.585	0.869	7.6	1.8	13.4	13.2	8.7	17.6
20	03-04-25	100	0.754	10.4	0.1103	14.6	0.729	0.588	0.871	7.7	1.8	13.6	13.2	8.7	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: **126**

Dilution preparation information:							***Comments:***
KCl Stock INSS number:			INSS 2357				
Stock preparation:			50 g KCl/L: Dissolve 50 g KCl in 1-L deionized water.				
Dilution prep (mg/L)	300	450	600	750	900	1050	
Stock volume (mL)	12	18	24	30	36	42	
Diluent volume (mL)	1988	1982	1976	1970	1964	1958	
Total volume (mL)	2000	2000	2000	2000	2000	2000	

Test organism information:		***Test information:***	
Organism source:	In-house culture	Randomizing template:	Yellow
Age:	< 24-hours old	Incubator number and shelf location:	7B
Spawn date:	02-25-25	*Artemia* CHM number:	CHM1294
Hatch dates and times:	03-03-25 1430 TO 03-04-25 0455	***Drying information for weight determination:***	
Transfer vessel information:	pH = 8.12 S.U. Temperature = 24.0 °C	Date / Time in oven:	03-11-25 0600
		*Initial oven temperature:	60°C
Average transfer volume:	< 0.25 mL	Date / Time out of oven:	03-12-25 0600
		*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	03-04-25	0500	[illegible]	1420	[illegible]	0710	[illegible]	02-26-25 A
1	03-05-25	0500	[illegible]	1400	[illegible]	0710	[illegible]	↓
2	03-06-25	0500	[illegible]	1315	[illegible]	0711	[illegible]	03-03-26 A
3	03-07-25	0500	[illegible]	1100	[illegible]	0700	[illegible]	↓
4	03-08-25	0530	[illegible]	1210	[illegible]	0730	[illegible]	03-06-25 A
5	03-09-25	0530	[illegible]	1130	[illegible]	0730	[illegible]	↓
6	03-10-25	0500	[illegible]	1100	[illegible]	0700	[illegible]	↓
7	03-11-25					0513	[illegible]	

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)	829.0
Average weight per initial larvae:	0.754		**NOEC** (mg/L KCl)	600
Average weight per surviving larvae:	0.754	≥ 0.25 mg/larvae	**LOEC** (mg/L KCl)	750
			ChV (mg/L KCl)	670.8
			IC_{25} (mg/L KCl)	678.6

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **126**

Survival and Growth Data

Day	Control				300 mg KCl/L				450 mg KCl/L			
	A	B	C	D	E	F	G	H	I	J	K	L
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10
*A = Pan weight (mg) Tray color code: Light pink Analyst: XL Date: 02-19-25	14.15	14.30	12.53	11.17	14.26	12.15	13.08	13.28	14.36	15.52	10.42	12.74
*B = Pan + Larvae weight (mg) Analyst: XL Date: 03-13-25	20.84	21.54	20.20	19.71	23.21	21.09	22.73	22.01	21.59	23.74	18.38	21.04
C = Larvae weight (mg) = B – A Analyst: K	6.69	7.24	7.67	8.54	8.95	8.94	9.65	8.73	7.23	8.22	7.96	8.30
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: K	0.669	0.724	0.767	0.854	0.895	0.894	0.965	0.873	0.723	0.822	0.796	0.830
Average weight per initial number of larvae (mg)	0.754				0.901				0.793			
Percent reduction from control (%)							-20.3%				-5.2%	

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

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

Comments:

Environmental Testing Solutions, Inc.

Species: ***Pimephales promelas*** PpKClCR Test Number: **126**

Survival and Growth Data

Day	600 mg KCl/L				750 mg KCl/L				900 mg KCl/L			
	M	N	O	P	Q	R	S	T	U	V	W	X
0	10	10	10	10	10	10	10	10	10	10	10	10
1	10	10	10	10	10	10	10	10	7 3d	7 3d	7 3d	7 3d
2	10	10	10	10	9 1d	9 1d	9 1d	9 1d	6 1d	6 1d	5 2d	5 2d
3	10	10	10	10	7 2d	8 1d	8 1d	9	4 2d	6	3 2d	5
4	10	9 1d	10	9 1d	7	8	8	9	4	5 1d	3	5
5	10	9	10	9	7	8	8	8 1d	4	5	3	5
6	10	9	10	9	7	8	8	8	3 1d	4 1d	3	4 1d
7	10	9	10	9	7	8	8	8	3	3 1d	3	3 1d
*A = Pan weight (mg) Tray color code: Light pink Analyst: XL Date: 02-19-25	12.40	12.30	14.34	14.27	13.35	13.89	13.52	13.60	12.67	13.80	13.58	12.14
*B = Pan + Larvae weight (mg) Analyst: XL Date: 03-13-25	20.06	18.31	22.31	20.83	18.19	19.73	19.13	19.22	15.41	16.30	16.13	14.01
C = Larvae weight (mg) = B – A Analyst: JL	7.66	6.01	7.97	6.56	4.84	5.84	5.61	5.62	2.74	2.50	2.55	1.87
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: JL	0.766	0.601	0.797	0.656	0.484	0.584	0.561	0.562	0.274	0.250	0.255	0.187
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.705	6.4%	0.548	27.3%	0.242	67.9%						

*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: **126**

Survival and Growth Data

Day	1050 mg KCl/L			
	Y	Z	AA	BB
0	10	10	10	10
1	4 6d	5 5d	5 5d	4 6d
2	3 4d	4 4d	4 4d	3 4d
3	2 3d	3 3d	4	3
4	1 2d	1 2d	1 3d	1 2d
5	1	1	1	1
6	1	1	0 1d	1 0d 03-20-25
7	1	1	0	1
*A = Pan weight (mg) Tray color code: Light pink Analyst: XL Date: 02-19-25	13.03	13.35	12.80	12.18
*B = Pan + Larvae weight (mg) Analyst: XL Date: 03-13-25	14.01	14.55	Z	12.98
C = Larvae weight (mg) = B – A Analyst: [illegible]	0.98	1.20	Z	0.80
Weight per initial number of larvae (mg) = C / Initial number of larvae Analyst: [illegible]	0.098	0.120	0	0.080
Average weight per initial number of larvae (mg) / Percent reduction from control (%)	0.075		90.17	

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

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

Comments:

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: 126

Test dates: March 04-11, 2025

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	14.15	20.84	6.69	0.669	0.754	10.4	0.669	100.0	0.754	10.4	Not applicable
	B	10	10	14.30	21.54	7.24	0.724			0.724				
	C	10	10	12.53	20.20	7.67	0.767			0.767				
	D	10	10	11.17	19.71	8.54	0.854			0.854				
300	E	10	10	14.26	23.21	8.95	0.895	0.907	4.4	0.895	100.0	0.907	4.4	-20.3
	F	10	10	12.15	21.09	8.94	0.894			0.894				
	G	10	10	13.08	22.73	9.65	0.965			0.965				
	H	10	10	13.28	22.01	8.73	0.873			0.873				
450	I	10	10	14.36	21.59	7.23	0.723	0.793	6.1	0.723	100.0	0.793	6.1	-5.2
	J	10	10	15.52	23.74	8.22	0.822			0.822				
	K	10	10	10.42	18.38	7.96	0.796			0.796				
	L	10	10	12.74	21.04	8.30	0.830			0.830				
600	M	10	10	12.40	20.06	7.66	0.766	0.740	7.5	0.766	95.0	0.705	13.1	6.4
	N	10	9	12.30	18.31	6.01	0.668			0.601				
	O	10	10	14.34	22.31	7.97	0.797			0.797				
	P	10	9	14.27	20.83	6.56	0.729			0.656				
750	Q	10	7	13.35	18.19	4.84	0.691	0.706	2.3	0.484	77.5	0.548	8.0	27.3
	R	10	8	13.89	19.73	5.84	0.730			0.584				
	S	10	8	13.52	19.13	5.61	0.701			0.561				
	T	10	8	13.60	19.22	5.62	0.703			0.562				
900	U	10	3	12.67	15.41	2.74	0.913	0.805	15.6	0.274	30.0	0.242	15.6	67.9
	V	10	3	13.80	16.30	2.50	0.833			0.250				
	W	10	3	13.58	16.13	2.55	0.850			0.255				
	X	10	3	12.14	14.01	1.87	0.623			0.187				
1050	Y	10	1	13.03	14.01	0.98	0.980	0.993	20.2	0.098	7.5	0.075	70.2	90.1
	Z	10	1	13.35	14.55	1.20	1.200			0.120				
	AA	10	0	0.00	0.00	0.00	0.000			0.000				
	BB	10	1	12.18	12.98	0.80	0.800			0.080				

Dunnett's MSD value: 0.1103 MSD = Minimum Significant Difference

PMSD: 14.6 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 Taylor

ETS Environmental Testing Solutions, Inc.

Statistical Analyses

Entered and Reviewed by Jim Summer

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	3/4/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/11/2025	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	0.9000	1.0000	0.9000
750	0.7000	0.8000	0.8000	0.8000
900	0.3000	0.3000	0.3000	0.3000
1050	0.1000	0.1000	0.0000	0.1000

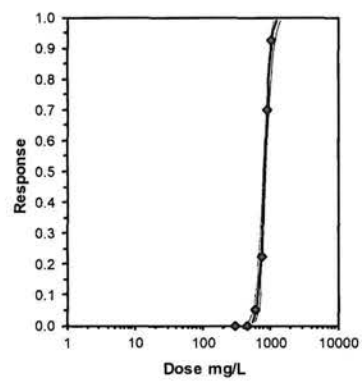
			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4			0	40
300	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
450	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	4	18.00	10.00	0	40
600	0.9500	0.9500	1.3305	1.2490	1.4120	7.072	4	14.00	10.00	2	40
*750	0.7750	0.7750	1.0782	0.9912	1.1071	5.379	4	10.00	10.00	9	40
*900	0.3000	0.3000	0.5796	0.5796	0.5796	0.000	4	10.00	10.00	28	40
*1050	0.0750	0.0750	0.2810	0.1588	0.3218	28.997	4	10.00	10.00	37	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.81792	0.896	-0.9185	1.55973
Equality of variance cannot be confirmed				

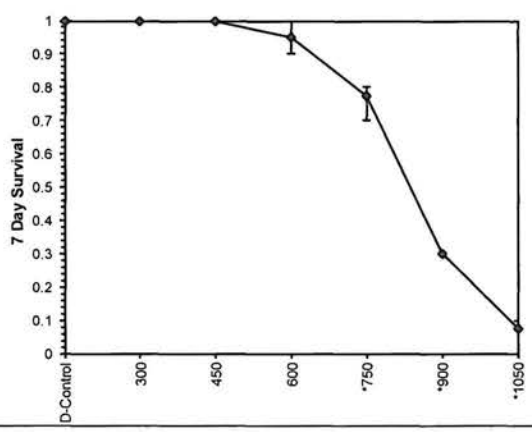
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	600	750	670.82	
Treatments vs D-Control				

					Maximum Likelihood-Probit						
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	13.4601	1.73458	10.0604	16.8599	0	1.26832	9.48773	0.86673	2.91856	0.07429	3
Intercept	-34.284	5.06978	-44.221	-24.347							
TSCR											

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	556.835	481.543	609.267
EC05	3.355	625.687	561.176	670.665
EC10	3.718	665.806	608.289	706.569
EC15	3.964	694.318	641.857	732.369
EC20	4.158	717.847	669.462	753.982
EC25	4.326	738.667	693.696	773.47
EC40	4.747	793.847	756.078	827.727
EC50	5.000	829.008	793.562	865.132
EC60	5.253	865.727	830.322	907.038
EC75	5.674	930.399	889.76	987.279
EC80	5.842	957.383	913.075	1022.66
EC85	6.036	989.827	940.309	1066.32
EC90	6.282	1032.21	974.907	1124.86
EC95	6.645	1098.4	1027.36	1218.98
EC99	7.326	1234.22	1131.17	1420.22



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:	3/4/2025	Test ID:	PpKCICR	Sample ID:	REF-Ref Toxicant
End Date:	3/11/2025	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	0.6690	0.7240	0.7670	0.8540
300	0.8950	0.8940	0.9650	0.8730
450	0.7230	0.8220	0.7960	0.8300
600	0.7660	0.6010	0.7970	0.6560
750	0.4840	0.5840	0.5610	0.5620
900	0.2740	0.2500	0.2550	0.1870
1050	0.0980	0.1200	0.0000	0.0800

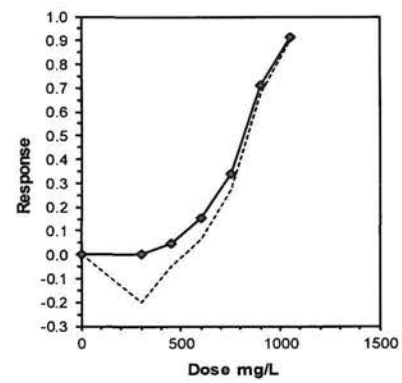
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7535	1.0000	0.7535	0.6690	0.8540	10.363	4				0.8301	1.0000
300	0.9068	1.2034	0.9068	0.8730	0.9650	4.426	4	-3.183	2.290	0.1103	0.8301	1.0000
450	0.7928	1.0521	0.7928	0.7230	0.8300	6.145	4	-0.815	2.290	0.1103	0.7928	0.9550
600	0.7050	0.9356	0.7050	0.6010	0.7970	13.052	4	1.007	2.290	0.1103	0.7050	0.8493
750	0.5478	0.7269	0.5478	0.4840	0.5840	7.997	4				0.5478	0.6598
900	0.2415	0.3205	0.2415	0.1870	0.2740	15.642	4				0.2415	0.2909
1050	0.0745	0.0989	0.0745	0.0000	0.1200	70.189	4				0.0745	0.0897

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9777	0.844	0.01503	-0.8042
Bartlett's Test indicates equal variances (p = 0.52)	2.24273	11.3449		

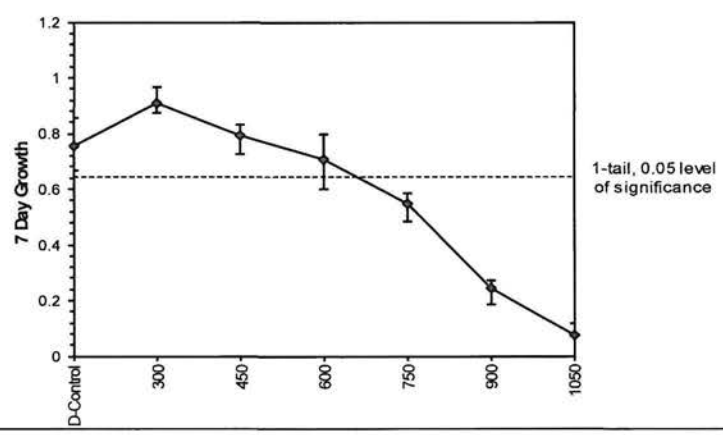
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	600	>600			0.11027	0.14634	0.02959	0.00464	0.00785	3, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	457.06	54.11	307.47	686.86	0.5202
IC10	528.01	53.04	366.61	687.65	0.0600
IC15	598.96	42.61	451.17	688.95	-0.5841
IC20	639.01	33.73	505.68	708.86	-0.6554
IC25	678.61	29.11	541.55	740.29	-1.0161
IC40	774.33	9.89	737.41	798.64	-0.4107
IC50	814.99	7.71	785.80	834.15	-0.3098



Dose-Response Plot



Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **126**

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	Em	Em	Em	Em	Em	N
Concentration	Parameter						
CONTROL, MHSW	pH (S.U.)	7.96	7.80	7.86	7.81	7.93	7.65
	Dissolved oxygen (mg/L)	8.1	7.8	8.0	8.0	8.1	6.3
	Conductivity (µmhos/cm)	313		308		309	
	Alkalinity (mg $CaCO_3$/L)	58				62	
	Hardness (mg $CaCO_3$/L)	88			23-09-25 N	88	
	Temperature (°C)	24.7	24.9	24.7	24.5	24.7	24.7
300 mg KCl/L	pH (S.U.)	7.99	7.79	7.91	7.78	8.02	7.52
	Dissolved oxygen (mg/L)	8.1	7.8	8.0	7.9	8.1	6.3
	Conductivity (µmhos/cm)	832		843		850	
	Temperature (°C)	24.6	25.1	24.8	24.8	24.6	24.9
450 mg KCl/L	pH (S.U.)	7.99	7.78	7.91	7.80	8.03	7.47
	Dissolved oxygen (mg/L)	8.2	7.8	8.0	7.9	8.2	6.1
	Conductivity (µmhos/cm)	1080		1110		1100	
	Temperature (°C)	24.6	25.0	24.8	24.6	24.6	24.7
600 mg KCl/L	pH (S.U.)	7.99	7.79	7.91	7.77	8.01	7.47
	Dissolved oxygen (mg/L)	8.2	7.8	8.0	7.9	8.2	5.9
	Conductivity (µmhos/cm)	1340		1370		1360	
	Temperature (°C)	24.6	25.2	24.6	24.6	24.6	24.7
750 mg KCl/L	pH (S.U.)	7.99	7.74	7.92	7.77	8.03	7.46
	Dissolved oxygen (mg/L)	8.2	7.9	8.0	7.9	8.2	5.9
	Conductivity (µmhos/cm)	1570		1620		1600	
	Temperature (°C)	24.8	25.2	24.6	24.9	24.8	24.6
900 mg KCl/L	pH (S.U.)	7.99	7.83	7.92	7.84	8.02	7.49
	Dissolved oxygen (mg/L)	8.2	7.8	8.0	7.9	8.2	6.3
	Conductivity (µmhos/cm)	1830		1870		1870	
	Temperature (°C)	24.8	24.8	24.8	24.5	24.8	24.6
1050 mg KCl/L	pH (S.U.)	8.00	7.82	7.92	7.81	8.03	7.50
	Dissolved oxygen (mg/L)	8.2	7.8	8.0	7.9	8.2	6.2
	Conductivity (µmhos/cm)	2100		2140		2170	
	Temperature (°C)	24.6	25.2	24.8	24.8	24.6	24.6
		Initial	Final	Initial	Final	Initial	Final

Environmental Testing Solutions, Inc.

Species: *Pimephales promelas* PpKClCR Test Number: **126**

		Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.)							
		3		4		5		6	
	Analyst	K	K	K	K	K	Em	Em	K
Concentration	Parameter								
CONTROL, MHSW	pH (S.U.)	8.09	7.49	8.01	7.60	8.11	7.81	7.90	7.47
	Dissolved oxygen (mg/L)	8.1	6.2	8.2	6.3	8.2	7.9	8.2	5.9
	Conductivity (µmhos/cm)	300		301		295		311	
	Alkalinity (mg $CaCO_3$/L)			62					
	Hardness (mg $CaCO_3$/L)	K 8-01-25 K		84				K 8-01-25 K	
	Temperature (°C)	24.8	24.9	24.9	24.7	24.8	24.8	24.7	24.7
300 mg KCl/L	pH (S.U.)	8.06	7.36	7.98	7.51	8.12	7.80	8.02	7.38
	Dissolved oxygen (mg/L)	8.0	6.2	8.2	6.4	8.2	8.0	8.1	6.0
	Conductivity (µmhos/cm)	816		804		826		850	
	Temperature (°C)	24.7	24.6	25.0	24.9	24.8	24.6	24.8	24.6
450 mg KCl/L	pH (S.U.)	8.06	7.37	7.98	7.52	8.09	7.80	8.01	7.38
	Dissolved oxygen (mg/L)	8.0	6.1	8.2	6.4	8.2	8.0	8.1	5.5
	Conductivity (µmhos/cm)	1050		1060		1080		1100	
	Temperature (°C)	24.7	24.6	24.8	24.6	24.9	24.6	24.8	24.6
600 mg KCl/L	pH (S.U.)	8.05	7.39	8.00	7.52	8.10	7.78	8.02	7.39
	Dissolved oxygen (mg/L)	8.1	6.1	8.2	6.4	8.2	8.0	8.2	5.8
	Conductivity (µmhos/cm)	1300		1300		1340		1370	
	Temperature (°C)	24.7	24.8	24.8	24.7	24.9	24.7	24.9	24.6
750 mg KCl/L	pH (S.U.)	8.05	7.41	8.00	7.52	8.10	7.75	8.01	7.38
	Dissolved oxygen (mg/L)	8.2	6.0	8.2	6.5	8.2	8.0	8.2	5.3
	Conductivity (µmhos/cm)	1540		1560		1610		1600	
	Temperature (°C)	24.7	24.6	24.8	24.7	24.8	24.9	24.8	24.6
900 mg KCl/L	pH (S.U.)	8.05	7.42	8.01	7.59	8.10	7.87	8.01	7.36
	Dissolved oxygen (mg/L)	8.2	6.4	8.2	6.7	8.2	8.0	8.2	5.0
	Conductivity (µmhos/cm)	1750		1800		1860		1860	
	Temperature (°C)	24.7	24.6	24.9	24.6	24.8	24.7	24.8	24.6
1050 mg KCl/L	pH (S.U.)	8.05	7.44	8.01	7.57	8.10	7.85	8.01	7.42
	Dissolved oxygen (mg/L)	8.2	6.0	8.2	6.3	8.2	8.0	8.2	5.8
	Conductivity (µmhos/cm)	2020		2040		2080		2130	
	Temperature (°C)	24.7	24.7	24.9	24.6	24.9	24.6	24.8	24.7
		Initial	Final	Initial	Final	Initial	Final	Initial	Final