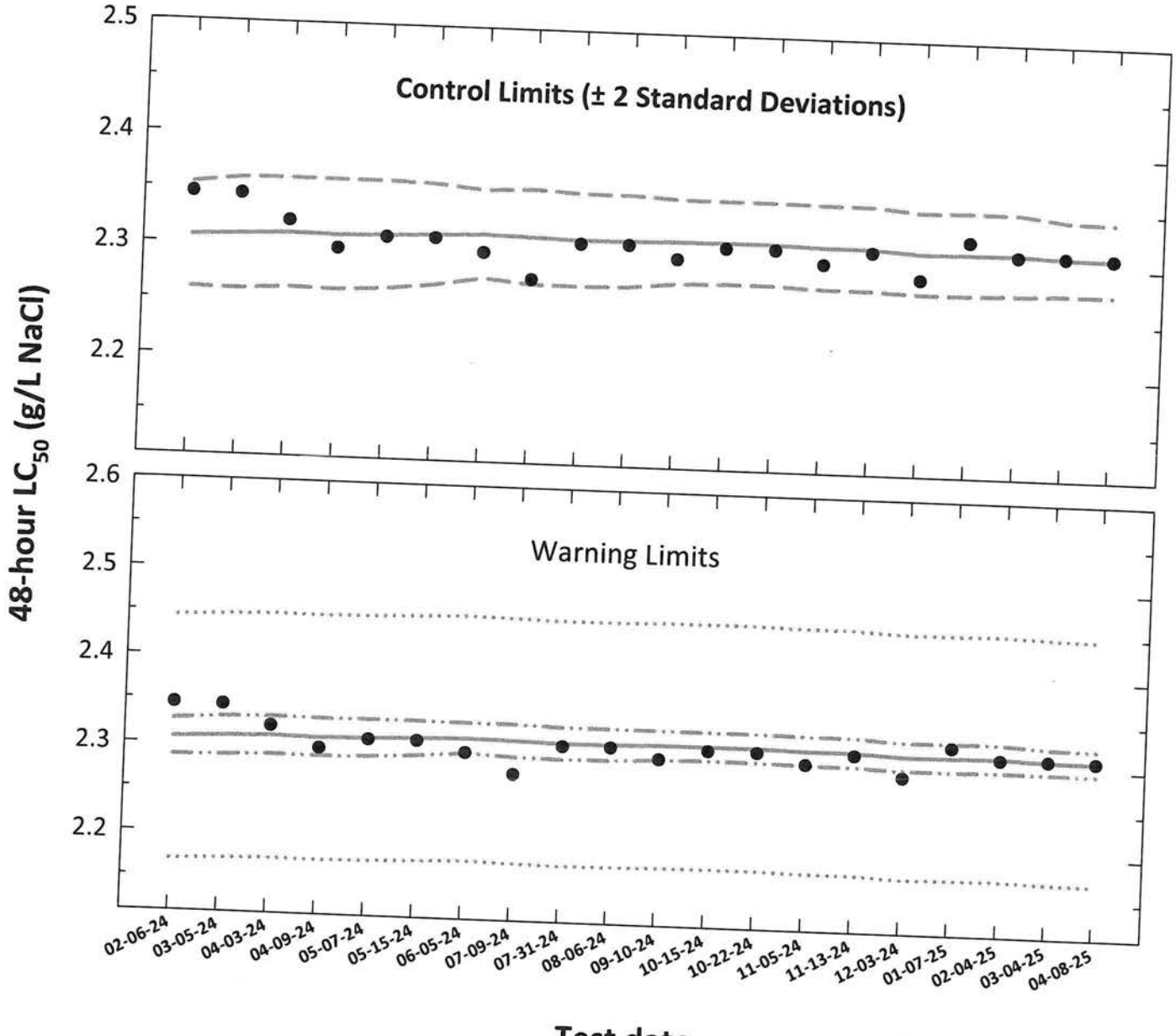
# *Ceriodaphnia dubia*
# Acute Reference Toxicant Control Chart
# Source: In-house Culture




- ● **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 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 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 |
| 10 | 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 |
| 11 | 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 |
| 12 | 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 |
| 13 | 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 |
| 14 | 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 |
| 15 | 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 |
| 16 | 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 |
| 17 | 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 |
| 18 | 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 |
| 19 | 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 |
| 20 | 04-08-25 | 2.3096 | 0.3635 | 0.3635 | 0.0031 | 2.3092 | 2.2770 | 2.3420 | 2.2953 | 2.3234 | 2.1707 | 2.4478 |

*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 $10^{th}$ percentile of CVs reported nationally by USEPA. ($S_{A.10}$ = 0.06).

**CV** = Coefficient of variation.

Entered and Reviewed by Jim Summer

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

## *Ceriodaphnia dubia* Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 424

### *Dilution Preparation:*

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

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

Stock solution INSS #: 2368

### *Chemical Analyses:*

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | EM | EM N | EM N |
| Control, MHSW | pH (S.U.) | 7.89 | 7.85 | 7.88 |
| | Dissolved oxygen (mg/L) | 0.2 | 0.1 | 0.1 |
| | Conductivity (µmhos/cm) | 297 | | |
| | Alkalinity (mg/L $CaCO_3$) | 59 | | |
| | Hardness (mg/L $CaCO_3$) | 89 | | |
| | Temperature (°C) | 24.9 | 25.2 | 25.2 |
| 1750 mg/L | pH (S.U.) | 7.96 | 7.86 | 7.88 |
| | Dissolved oxygen (mg/L) | 0.3 | 0.1 | 0.2 |
| | Conductivity (µmhos/cm) | 3300 | | |
| | Temperature (°C) | 25.0 | 25.0 | 25.0 |
| 2000 mg/L | pH (S.U.) | 7.96 | 7.86 | 7.88 |
| | Dissolved oxygen (mg/L) | 0.3 | 0.0 | 0.2 |
| | Conductivity (µmhos/cm) | 3720 | | |
| | Temperature (°C) | 25.0 | 25.2 | 25.0 |
| 2250 mg/L | pH (S.U.) | 7.97 | 7.86 | 7.90 |
| | Dissolved oxygen (mg/L) | 0.4 | 0.1 | 0.2 |
| | Conductivity (µmhos/cm) | 4100 | | |
| | Temperature (°C) | 25.0 | 25.2 | 25.0 |
| 2500 mg/L | pH (S.U.) | 7.97 | 7.87 | 7.90 |
| | Dissolved oxygen (mg/L) | 0.4 | 0.1 | 0.2 |
| | Conductivity (µmhos/cm) | 4500 | | |
| | Temperature (°C) | 25.0 | 25.3 | 25.3 |
| 2750 mg/L | pH (S.U.) | 7.98 | 7.88 | 7.90 |
| | Dissolved oxygen (mg/L) | 0.4 | 0.1 | 0.2 |
| | Conductivity (µmhos/cm) | 5050 | | |
| | Temperature (°C) | 25.0 | 25.0 | 25.0 |

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

### *Chemical analyses:*

| Parameter | Reporting limit | Method number | Meter | Serial numbe |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O G-2021 | YSI Model 52CE | 08A100271 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2021 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2021 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2021 | Not applicable | Not applicable |
| Temperature | 0.1°C | SM 2550B-2010 | Digital Thermometer | 13066465 |

ETS

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

***Ceriodaphnia dubia*** **Sodium Chloride Acute Reference Toxicant Test**

CdNaClAC # 424

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 04-08-25 | * 0530 | JL | 0747 | JL | 2B4 | RED | 04-02-25B |
| 24 | 04-09-25 | | | 0745 | JL | | | |
| 48 Termination | 04-10-25 | | | 0746 | 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): | 04-01-25 D |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 04-07-25 1500 TO 04-08-25 0530 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.94 |
| | Temperature (°C): 24.9 |

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

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

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

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | PROBIT |
| Lower 95% confidence limit (mg NaCl/L) | 2293.8 ~~2383.6~~ |
| Upper 95% confidence limit (mg NaCl/L) | 2383.6 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 2309.6 |

Comments:

Test Reviewed by: [signature]

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 4/8/2025 | Test ID: | CdNaCIAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 4/10/2025 | 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 | 0.4000 | 0.4000 | 0.6000 |
| 2500 | 0.4000 | 0.2000 | 0.2000 | 0.2000 |
| 2750 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

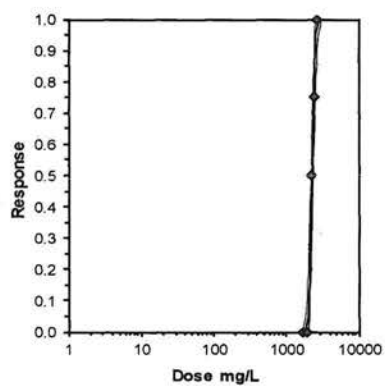
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1750 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2250 | 0.5000 | 0.5000 | 0.7854 | 0.6847 | 0.8861 | 14.802 | 4 | 10.00 | 10.00 | 10 | 20 |
| *2500 | 0.2500 | 0.2500 | 0.5189 | 0.4636 | 0.6847 | 21.301 | 4 | 10.00 | 10.00 | 15 | 20 |
| 2750 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.82032 | 0.868 | 0.91483 | 1.68855 |
| Equality of variance cannot be confirmed | | | | |

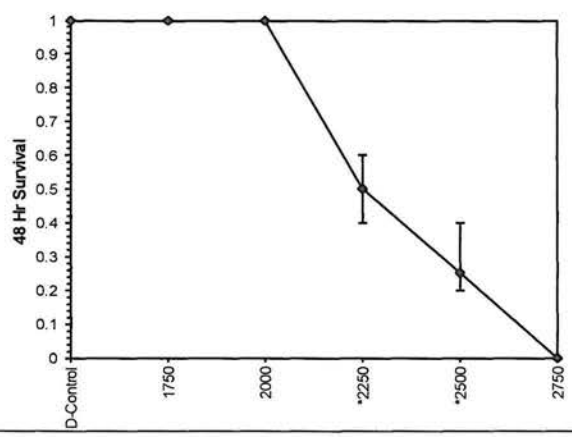
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2000 | 2250 | 2121.32 | |
| Treatments vs D-Control | | | | |

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

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




### Dose-Response Plot



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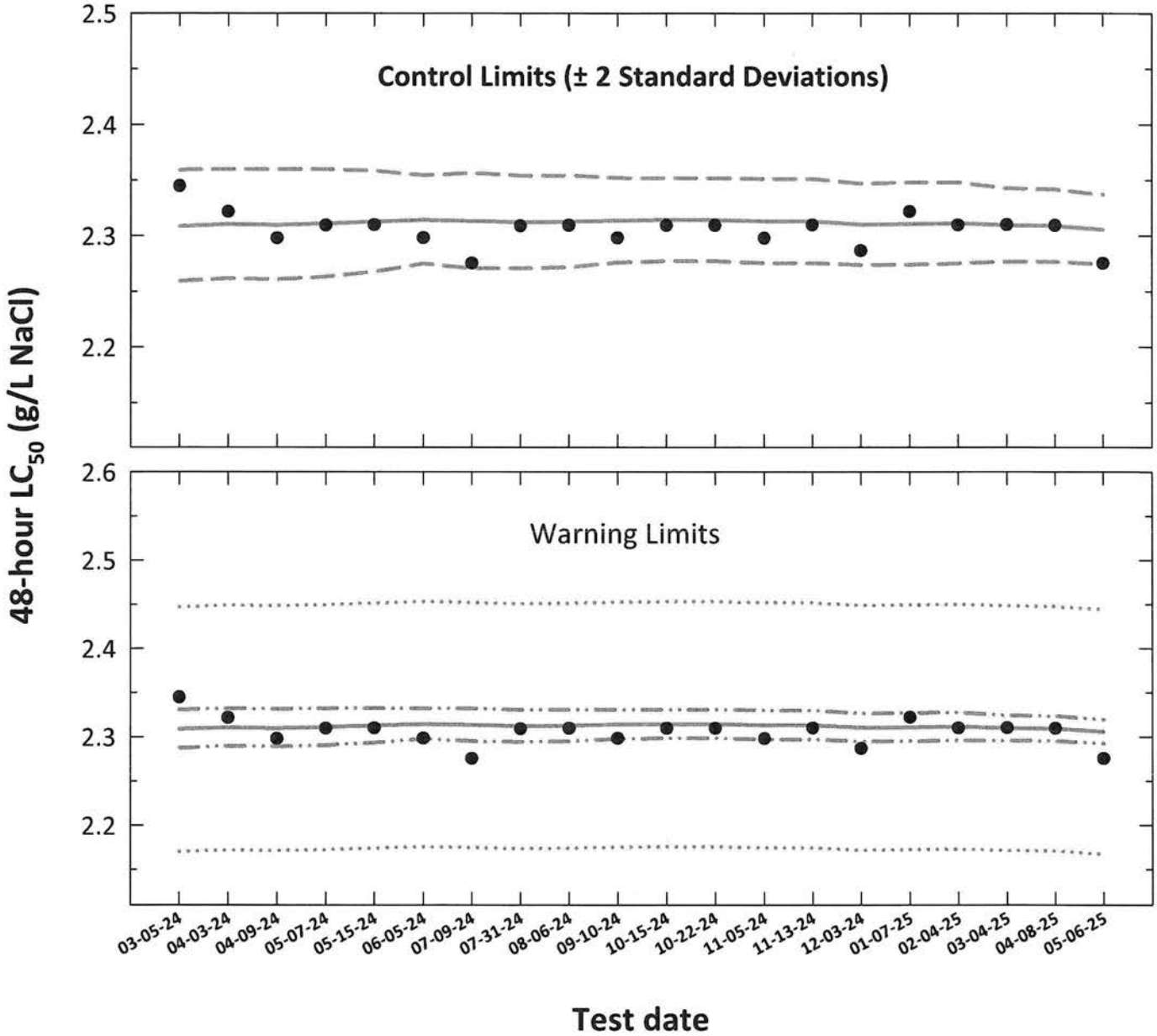


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- ····· **USEPA Warning Limits** (mean logarithmic $LC_{50} \pm S_{A.10}$ converted to anti-logarithmic values, $S_{A.10}$ = 10th percentile of CVs reported nationally by USEPA)

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# Acute Reference Toxicant Control Chart
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| 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 | 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 |
| 2 | 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 |
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| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 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 |
| 10 | 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 |
| 11 | 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 |
| 12 | 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 |
| 13 | 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 |
| 14 | 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 |
| 15 | 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 |
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| 17 | 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 |
| 18 | 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 |
| 19 | 04-08-25 | 2.3096 | 0.3635 | 0.3635 | 0.0031 | 2.3092 | 2.2770 | 2.3420 | 2.2953 | 2.3234 | 2.1707 | 2.4478 |
| 20 | 05-06-25 | 2.2755 | 0.3571 | 0.3628 | 0.0029 | 2.3057 | 2.2748 | 2.3371 | 2.2923 | 2.3194 | 2.1674 | 2.4441 |

*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.

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

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

**CV** = Coefficient of variation.

Entered and Reviewed by Jim Sumner

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

## *Ceriodaphnia dubia* Sodium Chloride Acute Reference Toxicant Test

**CdNaClAC #** 425

***Dilution Preparation:***

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

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

**Stock solution INSS #:** 2375

***Chemical Analyses:***

| | | Hours | | |
|---|---|---|---|---|
| | | 0 | 24 | 48 |
| Concentration | Analyst | N | EM | EM |
| Control, MHSW | pH (S.U.) | 8.13 | 7.84 | 7.84 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 296 | | |
| | Alkalinity (mg/L $CaCO_3$) | 62 | | |
| | Hardness (mg/L $CaCO_3$) | 88 | | |
| | Temperature (°C) | 25.0 | 25.3 | 25.0 |
| 1750 mg/L | pH (S.U.) | 8.09 | 7.86 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 3330 | | |
| | Temperature (°C) | 25.2 | 25.0 | 24.7 |
| 2000 mg/L | pH (S.U.) | 8.09 | 7.87 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 3800 | | |
| | Temperature (°C) | 25.2 | 25.0 | 25.0 |
| 2250 mg/L | pH (S.U.) | 8.08 | 7.88 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 4340 | | |
| | Temperature (°C) | 25.1 | 25.2 | 25.2 |
| 2500 mg/L | pH (S.U.) | 8.07 | 7.89 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 4720 | | |
| | Temperature (°C) | 24.8 | 25.2 | 25.2 |
| 2750 mg/L | pH (S.U.) | 8.07 | 7.88 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 5340 | | |
| | Temperature (°C) | 25.2 | 25.2 | 25.3 |

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

***Chemical analyses:***

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O 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 | 13044685 |

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 # 425

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 05-06-25 | * 0550 | JL | 0755 | JL | 233 | RED | 04-30-25B |
| 24 | 05-07-25 | | | 0750 | JL | | | |
| 48 Termination | 05-08-25 | | | 0752 | 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): | 04-29-25 D |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 05-05-25 1423 TO 05-06-25 0550 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 8.03 |
| | Temperature (°C): 25.0 |

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

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

| Hours | 2250 mg/L | | | | 2500 mg/L | | | | 2750 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 4 u | 5 | 5 | 5 | 4 1d | 4 1d | 3 2d | 3 2d | 4 1d | 3 2d |
| 48 Termination | 3 2d | 1 4d | 3 1d | 2 3d | 1 4d | 1 4d | 1 3d | 0 4d | 0 5d | 0 5d | 0 4d | 0 5d |
| Mean Survival | 45% | | | | 15% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | PROBIT |
| Lower 95% confidence limit (mg NaCl/L) | 2203.8 |
| Upper 95% confidence limit (mg NaCl/L) | 2345.1 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 2275.5 |

Comments:

Test Reviewed by: JL

ETS
Environmental Testing Solutions, Inc.

Entered and Reviewed by Jim Sumner

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/6/2025 | Test ID: | CdNaCIAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/8/2025 | 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 | 0.2000 | 0.6000 | 0.4000 |
| 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 | 0.4500 | 0.4500 | 0.7301 | 0.4636 | 0.8861 | 27.587 | 4 | 10.00 | 10.00 | 11 | 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 |

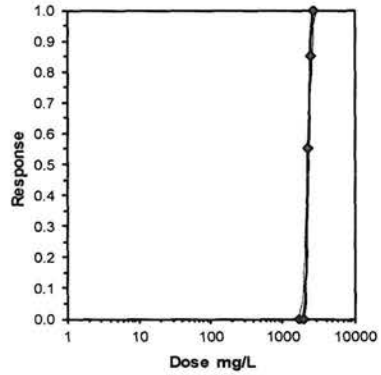
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.77497 | 0.868 | -1.2003 | 3.50343 |
| Equality of variance cannot be confirmed | | | | |

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

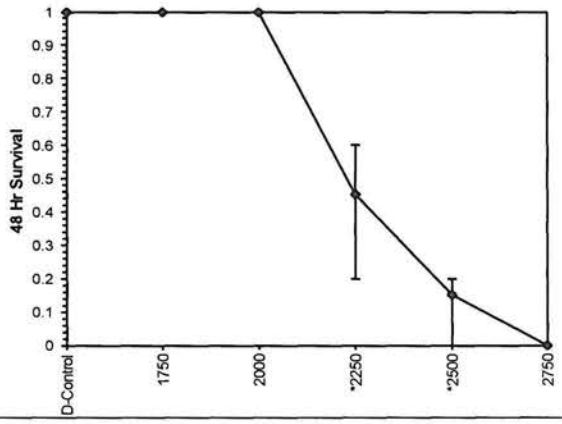
Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 32.3602 | 6.05281 | 20.4967 | 44.2237 | 0 | 2.60823 | 7.81472 | 0.45605 | 3.35708 | 0.0309 | 6 |
| Intercept | -103.64 | 20.3367 | -143.5 | -63.776 | | | | | | | |
| TSCR | | | | | | | | | | | |

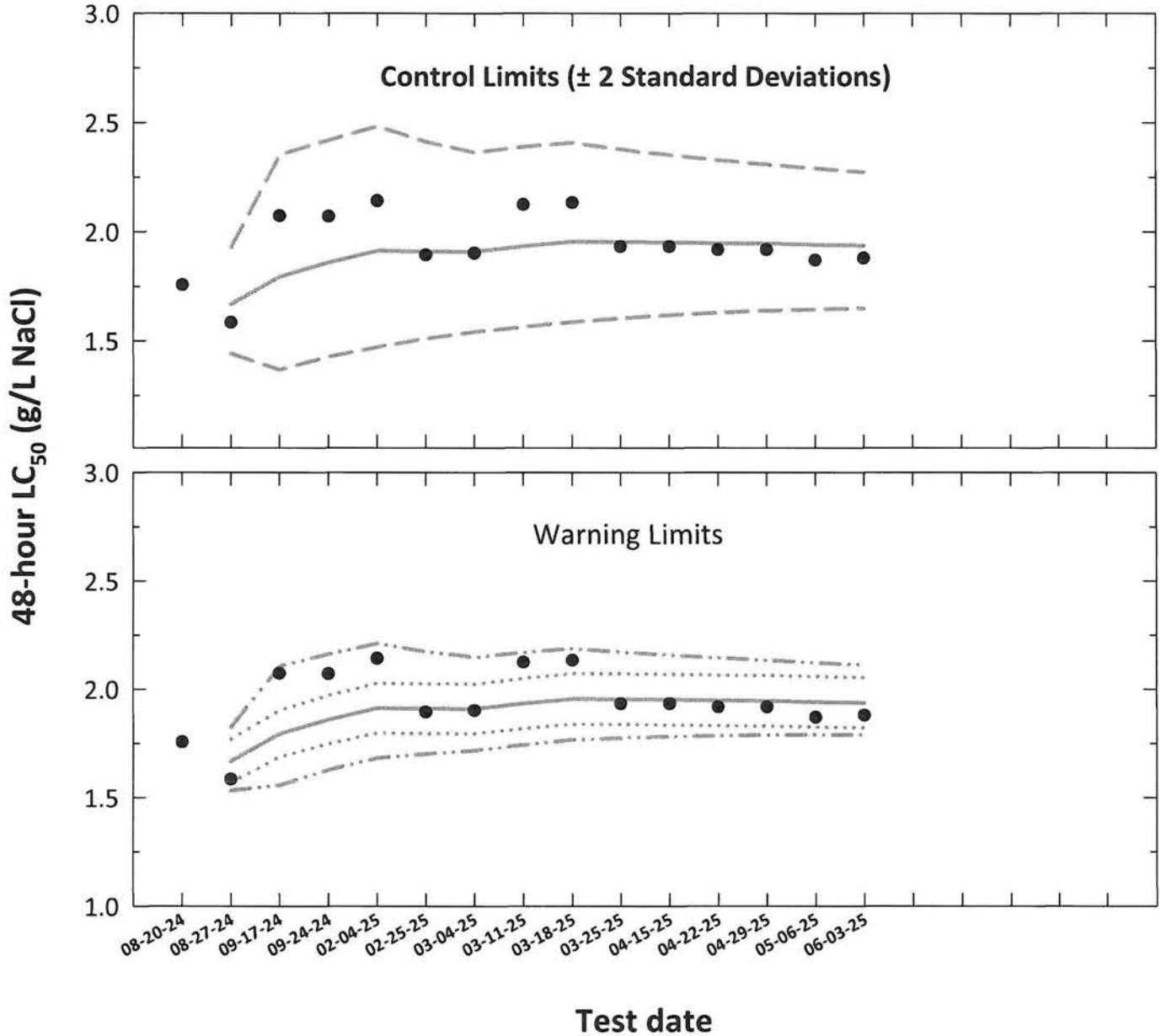
| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1928.38 | 1735.07 | 2032.02 |
| EC05 | 3.355 | 2024.2 | 1869.04 | 2110.02 |
| EC10 | 3.718 | 2077.21 | 1943.32 | 2154.27 |
| EC15 | 3.964 | 2113.75 | 1994.19 | 2185.65 |
| EC20 | 4.158 | 2143.26 | 2034.79 | 2211.76 |
| EC25 | 4.326 | 2168.9 | 2069.53 | 2235.21 |
| EC40 | 4.747 | 2234.88 | 2155.12 | 2300.32 |
| EC50 | 5.000 | 2275.53 | 2203.84 | 2345.14 |
| EC60 | 5.253 | 2316.92 | 2249.53 | 2395.23 |
| EC75 | 5.674 | 2387.4 | 2318.73 | 2490.34 |
| EC80 | 5.842 | 2415.96 | 2344.34 | 2531.76 |
| EC85 | 6.036 | 2449.69 | 2373.27 | 2582.28 |
| EC90 | 6.282 | 2492.79 | 2408.71 | 2648.92 |
| EC95 | 6.645 | 2558.07 | 2460.08 | 2753.24 |
| EC99 | 7.326 | 2685.17 | 2555.38 | 2964.81 |




### Dose-Response Plot



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




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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

| Test number | Test date | 48-hour $LC_{50}$ ToxCal Determination (g/L NaCl) | $Log_{10}$ Conversion | | | Anti-logarithmic Values (g/L NaCl) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 48-hour $LC_{50}$ | CT | S | CT | Control Limits | | Laboratory Calculated CV Warning Limits | | 10th Percentile CV Warning Limits | |
| | | | | | | | CT - 2S | CT + 2S | CT - 2CV | CT + 2CV | CT - $S_{A.10}$ | CT + $S_{A.10}$ |
| 1 | 08-20-24 | 1.7569 | 0.2447 | | | | | | | | | |
| 2 | 08-27-24 | 1.5847 | 0.2000 | 0.2224 | 0.0317 | 1.6686 | 1.4422 | 1.9306 | 1.5329 | 1.8256 | 1.5685 | 1.7687 |
| 3 | 09-17-24 | 2.0733 | 0.3167 | 0.2538 | 0.0589 | 1.7938 | 1.3679 | 2.3525 | 1.5564 | 2.1053 | 1.6862 | 1.9015 |
| 4 | 09-24-24 | 2.0711 | 0.3162 | 0.2694 | 0.0573 | 1.8595 | 1.4281 | 2.4211 | 1.6275 | 2.1615 | 1.7479 | 1.9710 |
| 5 | 02-04-25 | 2.1422 | 0.3309 | 0.2817 | 0.0567 | 1.9129 | 1.4730 | 2.4841 | 1.6829 | 2.2115 | 1.7981 | 2.0276 |
| 6 | 02-25-25 | 1.8940 | 0.2774 | 0.2810 | 0.0508 | 1.9097 | 1.5115 | 2.4128 | 1.7012 | 2.1732 | 1.7951 | 2.0243 |
| 7 | 03-04-25 | 1.9019 | 0.2792 | 0.2807 | 0.0464 | 1.9086 | 1.5417 | 2.3628 | 1.7163 | 2.1466 | 1.7941 | 2.0231 |
| 8 | 03-11-25 | 2.1247 | 0.3273 | 0.2865 | 0.0460 | 1.9344 | 1.5653 | 2.3905 | 1.7436 | 2.1701 | 1.8183 | 2.0504 |
| 9 | 03-18-25 | 2.1340 | 0.3292 | 0.2913 | 0.0453 | 1.9556 | 1.5874 | 2.4092 | 1.7673 | 2.1875 | 1.8382 | 2.0729 |
| 10 | 03-25-25 | 1.9328 | 0.2862 | 0.2908 | 0.0427 | 1.9533 | 1.6044 | 2.3781 | 1.7746 | 2.1708 | 1.8361 | 2.0705 |
| 11 | 04-15-25 | 1.9328 | 0.2862 | 0.2904 | 0.0406 | 1.9514 | 1.6189 | 2.3522 | 1.7810 | 2.1568 | 1.8343 | 2.0685 |
| 12 | 04-22-25 | 1.9184 | 0.2829 | 0.2897 | 0.0387 | 1.9486 | 1.6303 | 2.3292 | 1.7853 | 2.1439 | 1.8317 | 2.0656 |
| 13 | 04-29-25 | 1.9184 | 0.2829 | 0.2892 | 0.0371 | 1.9463 | 1.6404 | 2.3093 | 1.7891 | 2.1328 | 1.8295 | 2.0631 |
| 14 | 05-06-25 | 1.8700 | 0.2718 | 0.2880 | 0.0360 | 1.9407 | 1.6444 | 2.2905 | 1.7881 | 2.1209 | 1.8243 | 2.0572 |
| 15 | 06-03-25 | 1.8799 | 0.2741 | 0.2870 | 0.0349 | 1.9366 | 1.6494 | 2.2738 | 1.7883 | 2.1107 | 1.8204 | 2.0528 |
| 16 | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | |
| 18 | | | | | | | | | | | | |
| 19 | | | | | | | | | | | | |
| 20 | | | | | | | | | | | | |

*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 $10^{th}$ percentile of CVs reported nationally by USEPA. ($S_{A.10}$ = 0.06).

**CV** = Coefficient of variation.

# 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 #** 1

***Dilution Preparation:***

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2294

***Chemical Analyses:***

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | JP | JP | JP |
| Control, MHSW | pH (S.U.) | 7.51 | 7.41 | 7.28 |
| | Dissolved oxygen (mg/L) | 7.6 | 7.8 | 8.1 |
| | Conductivity (µmhos/cm) | 304 | | |
| | Alkalinity (mg/L $CaCO_3$) | ~~88~~ 61 | | |
| | Hardness (mg/L $CaCO_3$) | 88 | | |
| | Temperature (°C) | 24.7 | 24.1 | 25.2 |
| 1000 mg/L | pH (S.U.) | 7.74 | 7.56 | 7.54 |
| | Dissolved oxygen (mg/L) | 7.7 | 7.8 | 8.0 |
| | Conductivity (µmhos/cm) | 2130 | | |
| | Temperature (°C) | 24.2 | ~~24.3~~ 24.3 | 25.5 |
| 1500 mg/L | pH (S.U.) | 7.75 | 7.57 | 7.56 |
| | Dissolved oxygen (mg/L) | 7.6 | 7.9 | 8.2 |
| | Conductivity (µmhos/cm) | 3020 | | |
| | Temperature (°C) | 24.5 | 24.1 | 25.6 |
| 2000 mg/L | pH (S.U.) | 7.74 | 7.59 | 7.59 |
| | Dissolved oxygen (mg/L) | 7.8 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 3940 | | |
| | Temperature (°C) | 24.7 | 24.1 | 25.5 |
| 2500 mg/L | pH (S.U.) | 7.73 | 7.59 | 7.59 |
| | Dissolved oxygen (mg/L) | 7.7 | 8.0 | 8.2 |
| | Conductivity (µmhos/cm) | 4780 | | |
| | Temperature (°C) | 25.0 | 24.1 | 25.5 |
| 3000 mg/L | pH (S.U.) | 7.74 | 7.60 | |
| | Dissolved oxygen (mg/L) | 7.9 | 8.0 | |
| | Conductivity (µmhos/cm) | 5590 | | |
| | Temperature (°C) | 25.1 | 24.0 | |

JP 08-22-24

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

***Chemical analyses:***

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O 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 | 1306644 |

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 # 1

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 08-20-24 | *0839 | JP | 1053 | JP | 2 E | Rpd | 08-4-24 |
| 24 | 08-21-24 | | | 1051 | JP | | | |
| 48 Termination | 08-22-24 | | | 1051 | JP | | | |

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

*Test Organism Information:*

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 08-13-24 A and B |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 08-19-24 1350 to 08-20-24 0839<br>08-20-24 ~~0839~~ JP ~~0839~~ JP 08-20-24 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.42 |
| | Temperature (°C): 24.6°C |

*Survival Data* (number of living organisms):

| Hours | Control | | | | 1000 mg/L | | | | 1500 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 5 | 5 | ~~5~~ 6 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 6 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 48 Termination | 5 | 5 | 6 | 5 | 5 | 5 | 5 | 5 | 3d | 5 | 5 | 5 |
| Mean Survival | 100% | | | | 100% | | | | 90% | | | |

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 1d | 3d | 4d | 2d | 0d | 0d | 0d | 0d |
| 48 Termination | 0d | 2d | 0d | 1d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean Survival | 15% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

*Statistics:*

| | |
|---|---|
| Method | Probit (1640.9) |
| Lower 95% confidence limit (mg NaCl/L) | ~~1649.8~~ JP 1624.28.29 |
| Upper 95% confidence limit (mg NaCl/L) | 1874.1 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1756.9 |

Comments:

Test Reviewed by: JP

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 8/20/2024 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 8/22/2024 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | NACL-Sodium chloride |
| Sample Date: | | Protocol: | ACUTE-EPA-821-R-02-012 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 0.2000 | 0.6000 | 0.8000 | 0.4000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

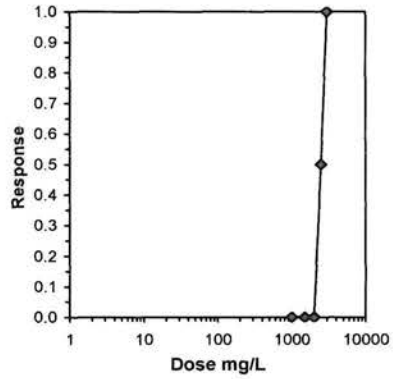
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3503 | 1.3453 | 1.3652 | 0.739 | 4 | | | 0 | 21 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 16.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 16.00 | 10.00 | 0 | 20 |
| 2000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 16.00 | 10.00 | 0 | 20 |
| *2500 | 0.5000 | 0.5000 | 0.7854 | 0.4636 | 1.1071 | 35.048 | 4 | 10.00 | 10.00 | 10 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.62206 | 0.868 | 0.00013 | 7.35313 |
| Equality of variance cannot be confirmed | | | | |

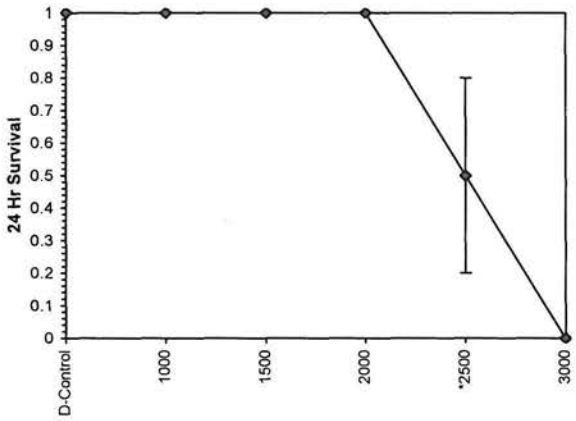
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2000 | 2500 | 2236.07 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2474.62 | 2364.94 | 2589.38 |
| 5.0% | 2477.14 | 2355.34 | 2605.24 |
| 10.0% | 2479.67 | 2342.53 | 2624.84 |
| 20.0% | 2484.74 | 2301.12 | 2683.01 |
| Auto-0.0% | 2474.62 | 2364.94 | 2589.38 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 8/20/2024 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 8/22/2024 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | NACL-Sodium chloride |
| Sample Date: | | Protocol: | ACUTE-EPA-821-R-02-012 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 0.6000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.0000 | 0.4000 | 0.0000 | 0.2000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | To |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Nun |
| D-Control | 1.0000 | 1.0000 | 1.3503 | 1.3453 | 1.3652 | 0.739 | 4 | | | 0 | |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 16.00 | 10.00 | 0 | |
| 1500 | 0.9000 | 0.9000 | 1.2305 | 0.8861 | 1.3453 | 18.660 | 4 | 14.50 | 10.00 | 2 | |
| *2000 | 0.1500 | 0.1500 | 0.3998 | 0.2255 | 0.6847 | 55.174 | 4 | 10.00 | 10.00 | 17 | |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | |

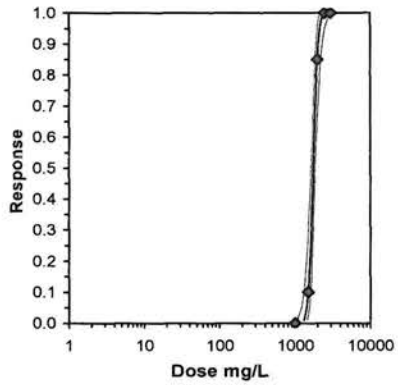
| Auxiliary Tests | Statistic | Critical | Skew | Ku |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.8928 | 0.844 | -0.6199 | 1.86 |
| Equality of variance cannot be confirmed | | | | |

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

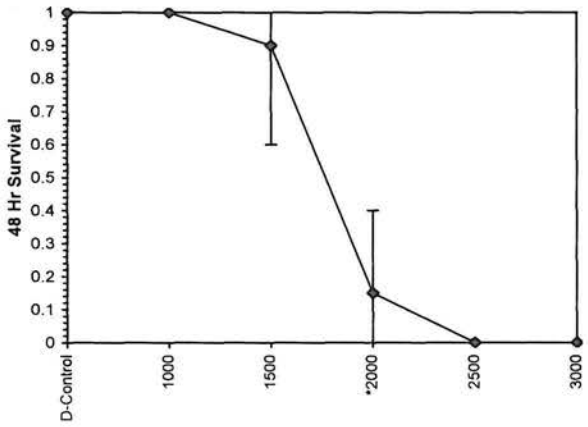
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | It |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 18.8728 | 3.85478 | 11.3174 | 26.4281 | 0 | 0.0457 | 7.81472 | 0.99744 | 3.24474 | 0.05299 | : |
| Intercept | -56.237 | 12.527 | -80.79 | -31.684 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1322.75 | 1071.12 | 1460.24 |
| EC05 | 3.355 | 1437.44 | 1224.27 | 1557.35 |
| EC10 | 3.718 | 1502.58 | 1312.72 | 1614.13 |
| EC15 | 3.964 | 1548.2 | 1374.65 | 1655.2 |
| EC20 | 4.158 | 1585.44 | 1424.83 | 1689.92 |
| EC25 | 4.326 | 1618.1 | 1468.29 | 1721.51 |
| EC40 | 4.747 | 1703.41 | 1577.34 | 1811.1 |
| EC50 | 5.000 | 1756.89 | 1640.79 | 1874.07 |
| EC60 | 5.253 | 1812.04 | 1701.33 | 1945.47 |
| EC75 | 5.674 | 1907.58 | 1794.83 | 2084.19 |
| EC80 | 5.842 | 1946.88 | 1829.86 | 2146.02 |
| EC85 | 6.036 | 1993.7 | 1869.63 | 2222.7 |
| EC90 | 6.282 | 2054.23 | 1918.56 | 2325.92 |
| EC95 | 6.645 | 2147.33 | 1989.92 | 2492.2 |
| EC99 | 7.326 | 2333.51 | 2123.79 | 2846.48 |




### Dose-Response Plot



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 #** 2

***Dilution Preparation:***

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2294

***Chemical Analyses:***

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | AP | AP | AP |
| Control, MHSW | pH (S.U.) | 7.06 | 7.35 | 7.26 |
| | Dissolved oxygen (mg/L) | 7.6 | 7.6 | 7.6 |
| | Conductivity (µmhos/cm) | 303 | | |
| | Alkalinity (mg/L $CaCO_3$) | 64 | | |
| | Hardness (mg/L $CaCO_3$) | 87 | | |
| | Temperature (°C) | 25.5 | 24.8 | 25.6 |
| 1000 mg/L | pH (S.U.) | 7.42 | 7.61 | 7.55 |
| | Dissolved oxygen (mg/L) | 7.5 | 7.6 | 7.9 |
| | Conductivity (µmhos/cm) | 2100 | | |
| | Temperature (°C) | 25.6 | 24.9 | 25.7 |
| 1500 mg/L | pH (S.U.) | 7.46 | 7.66 | 7.57 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.7 | 7.8 |
| | Conductivity (µmhos/cm) | 3100 | | |
| | Temperature (°C) | 25.6 | 24.8 | 25.0 |
| 2000 mg/L | pH (S.U.) | 7.49 | 7.64 | 7.59 |
| | Dissolved oxygen (mg/L) | 7.7 | 7.8 | 8.0 |
| | Conductivity (µmhos/cm) | 3940 | | |
| | Temperature (°C) | 25.4 | 24.3 | 25.2 |
| 2500 mg/L | pH (S.U.) | 7.50 | 7.66 | 7.61 |
| | Dissolved oxygen (mg/L) | 8.0 | 8.2 | 8.0 |
| | Conductivity (µmhos/cm) | 4870 | | |
| | Temperature (°C) | 25.7 | 24.8 | 25.5 |
| 3000 mg/L | pH (S.U.) | 7.52 | 7.67 | |
| | Dissolved oxygen (mg/L) | 7.9 | 8.2 | |
| | Conductivity (µmhos/cm) | 5750 | | |
| | Temperature (°C) | 25.7 | 24.9 | AP 08-29-24 |

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

***Chemical analyses:***

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O 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 | 13066 |

# 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 # 2

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 08-27-24 | *0949 | JP | 1320 | JP | 2B | Green | 08-26-24 |
| 24 | 08-28-24 | | | 1315 | JP | | | |
| 48 Termination | 08-29-24 | | | 1329 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 08-20-24 A & B |
| Age: | < 24-hours old |
| Date and time organisms were born between: | between 08-26-24 1427 and 08-27-24 0944 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl Information: | pH (S.U.): 6.93 |
| | Temperature (°C): 25.7 °C |

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

| Hours | Control | | | | 1000 mg/L | | | | 1500 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 48 Termination | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 2 3d | 3 2d | 3 2d | 5 |
| Mean Survival | 100% | | | | 100% | | | | 65% | | | |

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 3 2d | 5 JP 08-29-24 | 5 | 4 1d | 4 1d | 4 1d | 0 5d | 4 1d | 0 5d | 0 5d | 0 5d | 0 5d |
| 48 Termination | 0 3d | 3 2d 0 5d | 1 4d | 0 4d | 0 | 0 4d | 0 | 0 4d | 0 | 0 | 0 | 0 |
| Mean Survival | 5% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Probit |
| Lower 95% confidence limit (mg NaCl/L) | 1456.7 |
| Upper 95% confidence limit (mg NaCl/L) | 1701.8 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1584.1 |

Comments:

Test Reviewed by: JP

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 8/27/2024 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 8/29/2024 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | NACL-Sodium chloride |
| Sample Date: | | Protocol: | ACUTE-EPA-821-R-02-012 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.6000 | 1.0000 | 1.0000 | 0.8000 |
| 2500 | 0.8000 | 0.8000 | 0.0000 | 0.8000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 0.8500 | 0.8500 | 1.1709 | 0.8861 | 1.3453 | 18.840 | 4 | 14.00 | 10.00 | 3 | 20 |
| *2500 | 0.6000 | 0.6000 | 0.8867 | 0.2255 | 1.1071 | 49.712 | 4 | 10.00 | 10.00 | 8 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

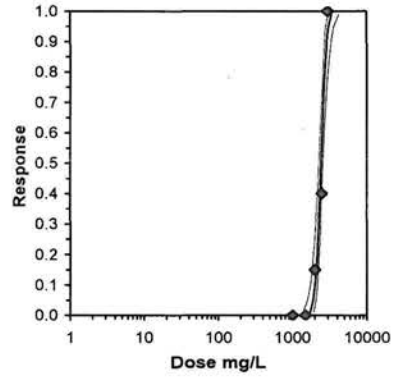
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.70769 | 0.868 | -2.0992 | 6.60427 |
| Equality of variance cannot be confirmed | | | | |

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

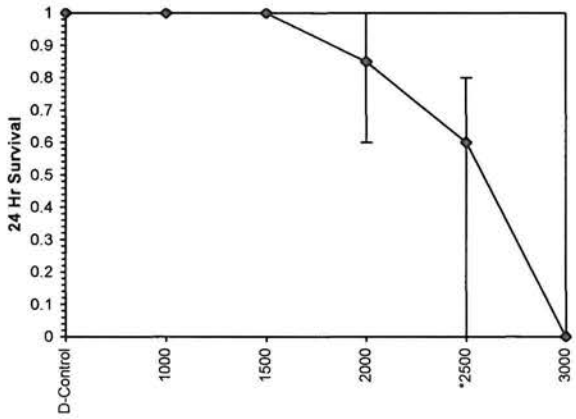
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 15.9565 | 3.1819 | 9.71995 | 22.193 | 0 | 5.06667 | 7.81472 | 0.16698 | 3.38646 | 0.06267 | 6 |
| Intercept | -49.036 | 10.7908 | -70.186 | -27.886 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1740.48 | 1378.39 | 1940.26 |
| EC05 | 3.355 | 1920.34 | 1613.23 | 2091 |
| EC10 | 3.718 | 2023.7 | 1752.02 | 2179.01 |
| EC15 | 3.964 | 2096.57 | 1850.61 | 2242.57 |
| EC20 | 4.158 | 2156.34 | 1931.34 | 2296.27 |
| EC25 | 4.326 | 2208.98 | 2001.84 | 2345.19 |
| EC40 | 4.747 | 2347.39 | 2180.44 | 2485.23 |
| EC50 | 5.000 | 2434.79 | 2284.4 | 2585.94 |
| EC60 | 5.253 | 2525.46 | 2382.61 | 2702.82 |
| EC75 | 5.674 | 2683.69 | 2532.3 | 2935.35 |
| EC80 | 5.842 | 2749.2 | 2588.22 | 3040.18 |
| EC85 | 6.036 | 2827.59 | 2651.94 | 3170.72 |
| EC90 | 6.282 | 2929.39 | 2730.91 | 3347.16 |
| EC95 | 6.645 | 3087.07 | 2847.43 | 3633.11 |
| EC99 | 7.326 | 3406.09 | 3070.26 | 4249.88 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 8/27/2024 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 8/29/2024 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | NACL-Sodium chloride |
| Sample Date: | | Protocol: | ACUTE-EPA-821-R-02-012 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 0.4000 | 0.6000 | 0.6000 | 1.0000 |
| 2000 | 0.0000 | 0.0000 | 0.2000 | 0.0000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.6500 | 0.6500 | 0.9505 | 0.6847 | 1.3453 | 29.432 | 4 | 12.00 | 10.00 | 7 | 20 |
| *2000 | 0.0500 | 0.0500 | 0.2850 | 0.2255 | 0.4636 | 41.771 | 4 | 10.00 | 10.00 | 19 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

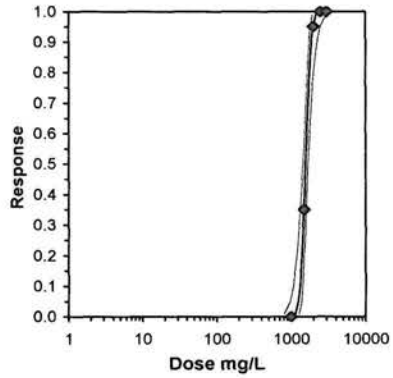
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.75091 | 0.844 | 1.43221 | 5.14157 |
| Equality of variance cannot be confirmed | | | | |

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

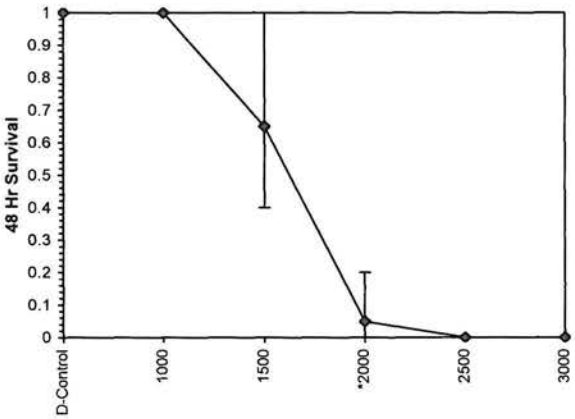
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 16.5316 | 4.09072 | 8.51378 | 24.5494 | 0 | 0.02427 | 7.81472 | 0.999 | 3.19996 | 0.06049 | 3 |
| Intercept | -47.9 | 13.13 | -73.635 | -22.166 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1146.13 | 817.357 | 1299.71 |
| EC05 | 3.355 | 1260.26 | 978.367 | 1391.75 |
| EC10 | 3.718 | 1325.67 | 1075.23 | 1445.55 |
| EC15 | 3.964 | 1371.71 | 1144.79 | 1484.52 |
| EC20 | 4.158 | 1409.44 | 1202.2 | 1517.57 |
| EC25 | 4.326 | 1442.64 | 1252.66 | 1547.87 |
| EC40 | 4.747 | 1529.79 | 1381.58 | 1636.11 |
| EC50 | 5.000 | 1584.74 | 1456.67 | 1701.79 |
| EC60 | 5.253 | 1641.65 | 1526.43 | 1781 |
| EC75 | 5.674 | 1740.83 | 1627.94 | 1946.81 |
| EC80 | 5.842 | 1781.83 | 1664.15 | 2023.99 |
| EC85 | 6.036 | 1830.84 | 1704.47 | 2121.44 |
| EC90 | 6.282 | 1894.43 | 1753.33 | 2254.92 |
| EC95 | 6.645 | 1992.76 | 1823.89 | 2474.39 |
| EC99 | 7.326 | 2191.18 | 1955.82 | 2957.62 |




### Dose-Response Plot



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

## *Ceriodaphnia dubia* Sodium Chloride Acute Reference Toxicant Test

**CdNaClAC #** 3

***Dilution Preparation:***

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2320

***Chemical Analyses:***

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | JP | JP | JP |
| Control, MHSW | pH (S.U.) | 7.55 | 7.32 | 7.26 |
| | Dissolved oxygen (mg/L) | 7.4 | 7.8 | 7.7 |
| | Conductivity (µmhos/cm) | 307 | | |
| | Alkalinity (mg/L $CaCO_3$) | 64 | | |
| | Hardness (mg/L $CaCO_3$) | 87 | | |
| | Temperature (°C) | 25 24.5 JP 09-17-24 | 24.4 | 24.5 |
| 1000 mg/L | pH (S.U.) | 7.72 | 7.56 | 7.51 |
| | Dissolved oxygen (mg/L) | 7.6 | 7.8 | 7.9 |
| | Conductivity (µmhos/cm) | 2010 | | |
| | Temperature (°C) | 24.4 | 24.7 | 24.5 |
| 1500 mg/L | pH (S.U.) | 7.73 | 7.60 | 7.57 |
| | Dissolved oxygen (mg/L) | 7.7 | 7.7 | 7.8 |
| | Conductivity (µmhos/cm) | 2910 | | |
| | Temperature (°C) | 24.3 | 24.6 | 24.1 |
| 2000 mg/L | pH (S.U.) | 7.73 | 7.60 | 7.58 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.9 | 8.0 |
| | Conductivity (µmhos/cm) | 3740 | | |
| | Temperature (°C) | 24.3 | 24.6 | 24.3 |
| 2500 mg/L | pH (S.U.) | 7.74 | 7.61 | 7.60 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.4 | 8.1 |
| | Conductivity (µmhos/cm) | 4640 | | |
| | Temperature (°C) | 24.5 | 24.2 | 24.2 |
| 3000 mg/L | pH (S.U.) | 7.74 | 7.62 | 7.59 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.6 | 8.1 |
| | Conductivity (µmhos/cm) | 5380 | | |
| | Temperature (°C) | 24.1 | 24.5 | 24.3 |

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measuremer only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on tl 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 | 13066 |

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

***Ceriodaphnia dubia*** **Sodium Chloride Acute Reference Toxicant Test**

CdNaClAC # 3

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 09-17-24 | *0830 | JP | 1102 | JP | 7B | Blue | 09-12-24 B |
| 24 | 09-18-24 | | | 1115 | JP | | | |
| 48 Termination | 09-20-24 | | | 1100 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 09-10-24 A+B |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 09-16-24 1341 and 09-17-24 0900 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.19 |
| | Temperature (°C): 24.1 |

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

| Hours | Control | | | | 1000 mg/L | | | | 1500 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 48 Termination | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 1d | 4 1d | 5 |
| Mean Survival | 100% | | | | 100% | | | | 90% | | | |

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 5 | 4 1d | 4 1d | 1 4d | 0 | 1 4d | 2 3d | 0 |
| 48 Termination | 2 3d | 4 1d | 5 | 5 | 0 5d | 1 3d | 0 4d | 0 1d | 0 | 0 1d | 0 2d | 0 |
| Mean Survival | 80% | | | | 5% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Spearman-Karber |
| Lower 95% confidence limit (mg NaCl/L) | 1936.7 |
| Upper 95% confidence limit (mg NaCl/L) | 2219.4 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 2073.3 |

Comments:

Test Reviewed by: [signature]

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/17/2024 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 9/20/2024 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | NACL-Sodium chloride |
| Sample Date: | | Protocol: | ACUTE-EPA-821-R-02-012 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 1.0000 | 0.8000 | 0.8000 | 0.2000 |
| 3000 | 0.0000 | 0.2000 | 0.4000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2500 | 0.7000 | 0.7000 | 1.0058 | 0.4636 | 1.3453 | 37.629 | 4 | 12.00 | 10.00 | 6 | 20 |
| *3000 | 0.1500 | 0.1500 | 0.3998 | 0.2255 | 0.6847 | 55.174 | 4 | 10.00 | 10.00 | 17 | 20 |

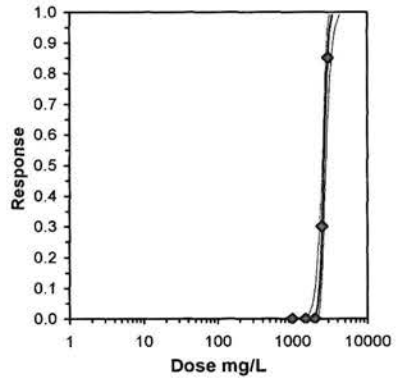
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.71602 | 0.884 | -1.262 | 6.32816 |
| Equality of variance cannot be confirmed | | | | |

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

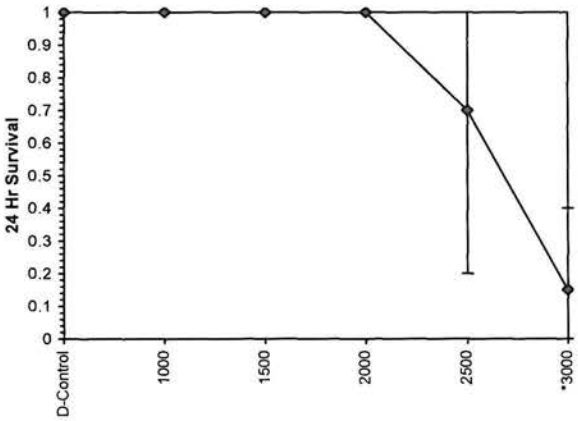
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 20.8799 | 4.82936 | 11.4144 | 30.3455 | 0 | 0.13925 | 7.81472 | 0.98674 | 3.42559 | 0.04789 | 3 |
| Intercept | -66.526 | 16.5485 | -98.961 | -34.091 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 2061.46 | 1648.32 | 2255.74 |
| EC05 | 3.355 | 2222.36 | 1884.58 | 2383.84 |
| EC10 | 3.718 | 2313.2 | 2021.61 | 2458.08 |
| EC15 | 3.964 | 2376.58 | 2117.79 | 2511.67 |
| EC20 | 4.158 | 2428.19 | 2195.77 | 2557.08 |
| EC25 | 4.326 | 2473.36 | 2263.19 | 2598.72 |
| EC40 | 4.747 | 2590.94 | 2430.19 | 2720.29 |
| EC50 | 5.000 | 2664.35 | 2523.69 | 2810.37 |
| EC60 | 5.253 | 2739.84 | 2608.7 | 2916.89 |
| EC75 | 5.674 | 2870.08 | 2732.51 | 3130.09 |
| EC80 | 5.842 | 2923.47 | 2777.45 | 3225.68 |
| EC85 | 6.036 | 2986.96 | 2828.06 | 3344.01 |
| EC90 | 6.282 | 3068.8 | 2890.05 | 3502.69 |
| EC95 | 6.645 | 3194.24 | 2980.37 | 3756.97 |
| EC99 | 7.326 | 3443.55 | 3149.93 | 4295.06 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/17/2024 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 9/20/2024 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | NACL-Sodium chloride |
| Sample Date: | | Protocol: | ACUTE-EPA-821-R-02-012 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 0.8000 | 0.8000 | 1.0000 |
| 2000 | 0.4000 | 0.8000 | 1.0000 | 1.0000 |
| 2500 | 0.0000 | 0.2000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

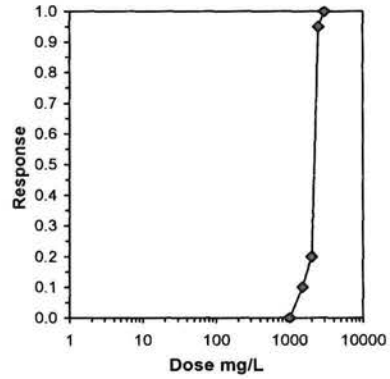
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.9000 | 0.9000 | 1.2262 | 1.1071 | 1.3453 | 11.212 | 4 | 14.00 | 10.00 | 2 | 20 |
| 2000 | 0.8000 | 0.8000 | 1.1206 | 0.6847 | 1.3453 | 27.799 | 4 | 14.00 | 10.00 | 4 | 20 |
| *2500 | 0.0500 | 0.0500 | 0.2850 | 0.2255 | 0.4636 | 41.771 | 4 | 10.00 | 10.00 | 19 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.85016 | 0.868 | -1.0936 | 3.8286 |
| Equality of variance cannot be confirmed | | | | |

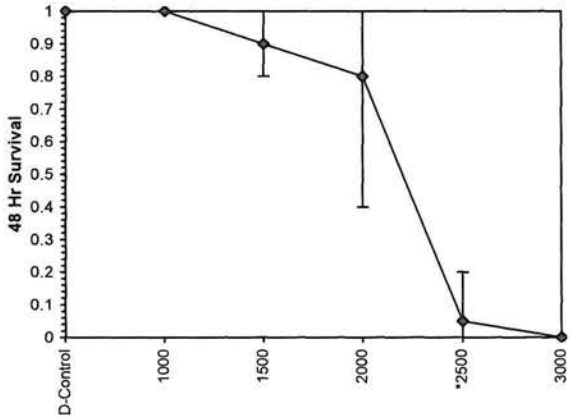
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2000 | 2500 | 2236.07 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2073.25 | 1936.72 | 2219.39 |
| 5.0% | 2113.89 | 1974.27 | 2263.37 |
| 10.0% | 2151.76 | 2020.34 | 2291.72 |
| 20.0% | 2186.72 | 2113.68 | 2262.3 |
| Auto-0.0% | 2073.25 | 1936.72 | 2219.39 |




### Dose-Response Plot



# 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 # L

### *Dilution Preparation:*

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

Stock solution INSS #: 2320

### *Chemical Analyses:*

| | | Hours | | |
|---|---|---|---|---|
| | | 0 | 24 | 48 |
| Concentration | Analyst | AP | AP | |
| Control, MHSW | pH (S.U.) | 7.49 | 7.32 | 7.44 |
| | Dissolved oxygen (mg/L) | ~~7.6~~ 7.9 | 7.2 | 6.9 |
| | Conductivity (µmhos/cm) | 325 | | |
| | Alkalinity (mg/L $CaCO_3$) | 58 | | |
| | Hardness (mg/L $CaCO_3$) | 88 | | |
| | Temperature (°C) | 24.9 | 25.2 | 25.6 |
| 1000 mg/L | pH (S.U.) | 7.66 | 7.55 | 7.61 |
| | Dissolved oxygen (mg/L) | 7.6 | 7.3 | 7.2 |
| | Conductivity (µmhos/cm) | 2160 | | |
| | Temperature (°C) | 24.5 | 25.2 | 25.7 |
| 1500 mg/L | pH (S.U.) | 7.68 | 7.57 | 7.63 |
| | Dissolved oxygen (mg/L) | 7.6 | 7.3 | 7.3 |
| | Conductivity (µmhos/cm) | 2980 | | |
| | Temperature (°C) | 24.9 | 25.2 | 25.5 |
| 2000 mg/L | pH (S.U.) | 7.68 | 7.59 | 7.64 |
| | Dissolved oxygen (mg/L) | ~~7.7~~ 7.2 | 7.5 | 7.6 |
| | Conductivity (µmhos/cm) | 3920 | | |
| | Temperature (°C) | 24.9 | 25.0 | 25.2 |
| 2500 mg/L | pH (S.U.) | 7.69 | 7.60 | 7.64 |
| | Dissolved oxygen (mg/L) | 7.8 | 7.7 | 7.6 |
| | Conductivity (µmhos/cm) | 4900 | | |
| | Temperature (°C) | 24.9 | 25.2 | 25.5 |
| 3000 mg/L | pH (S.U.) | 7.69 | 7.62 | 7.65 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.9 | 7.7 |
| | Conductivity (µmhos/cm) | 5780 | | |
| | Temperature (°C) | 24.5 | 25.2 | 25.2 |

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

### *Chemical analyses:*

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O 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 | 170664 |

# 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 # 4

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 09-24-24 | *0818 | JP | 1036 | JP | 2B | Blue | 0918-24 |
| 24 | 09-25-24 | | | 1030 | JP | | | |
| 48 Termination | 09-26-24 | | | 1030 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 09-17-24 A + B |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 09-23-24 1410 and 09-24-24 0913 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.35 |
| | Temperature (°C): 24.1 °C |

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

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

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 2 3d | 2 3d | 5 | 5 | 0 5d | 0 5d | 0 5d | 1 4d |
| 48 Termination | 4 1d | 3 2d | 3 2d | 4 1d | 0 2d | 0 2d | 0 5d | 0 5d | 0 | 0 | 0 | 0 1d |
| Mean Survival | 70% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Spearman-Karber |
| Lower 95% confidence limit (mg NaCl/L) | 1965.5 |
| Upper 95% confidence limit (mg NaCl/L) | 2182.4 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 2071.1 |

Comments:

Test Reviewed by:

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/24/2024 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 9/24/2024 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | NACL-Sodium chloride |
| Sample Date: | | Protocol: | ACUTE-EPA-821-R-02-012 | Test Species: | CD-Ceriodaphnia dubia |

Comments:

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 0.4000 | 0.4000 | 1.0000 | 1.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.2000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2500 | 0.7000 | 0.7000 | 1.0150 | 0.6847 | 1.3453 | 37.574 | 4 | 14.00 | 10.00 | 6 | 20 |
| *3000 | 0.0500 | 0.0500 | 0.2850 | 0.2255 | 0.4636 | 41.771 | 4 | 10.00 | 10.00 | 19 | 20 |

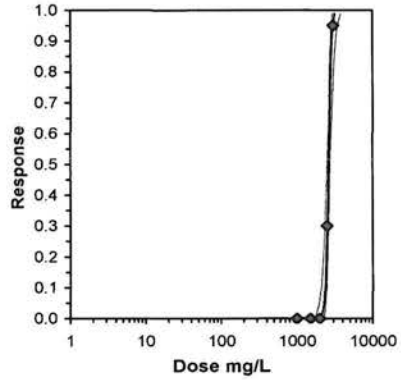
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.71655 | 0.884 | 0.07995 | 2.90237 |
| Equality of variance cannot be confirmed | | | | |

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

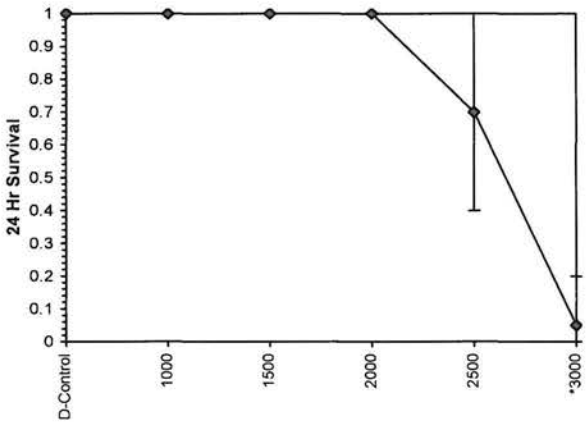
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 27.6657 | 6.71551 | 14.5033 | 40.8281 | 0 | 0.01469 | 7.81472 | 0.99953 | 3.41721 | 0.03615 | 3 |
| Intercept | -89.539 | 22.9599 | -134.54 | -44.538 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 2153.4 | 1785.37 | 2314.37 |
| EC05 | 3.355 | 2279.08 | 1983.23 | 2412.5 |
| EC10 | 3.718 | 2349.04 | 2095.36 | 2469.06 |
| EC15 | 3.964 | 2397.46 | 2172.99 | 2509.79 |
| EC20 | 4.158 | 2436.65 | 2235.31 | 2544.25 |
| EC25 | 4.326 | 2470.78 | 2288.78 | 2575.79 |
| EC40 | 4.747 | 2558.92 | 2419.79 | 2667.42 |
| EC50 | 5.000 | 2613.45 | 2492.5 | 2734.68 |
| EC60 | 5.253 | 2669.14 | 2558.3 | 2813.61 |
| EC75 | 5.674 | 2764.35 | 2653.03 | 2970.48 |
| EC80 | 5.842 | 2803.07 | 2686.88 | 3040.44 |
| EC85 | 6.036 | 2848.89 | 2724.62 | 3126.68 |
| EC90 | 6.282 | 2907.61 | 2770.32 | 3241.63 |
| EC95 | 6.645 | 2996.87 | 2835.98 | 3424.04 |
| EC99 | 7.326 | 3171.77 | 2956.94 | 3802.6 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 9/24/2024 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 9/26/2024 | Lab ID: | ETS-Envir. Testing Sol. | Sample Type: | NACL-Sodium chloride |
| Sample Date: | | Protocol: | ACUTE-EPA-821-R-02-012 | Test Species: | CD-Ceriodaphnia dubia |
| Comments: | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.8000 | 0.6000 | 0.6000 | 0.8000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

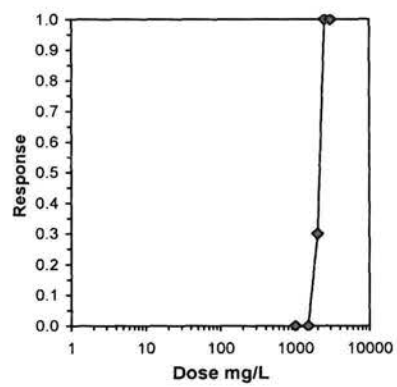
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2000 | 0.7000 | 0.7000 | 0.9966 | 0.8861 | 1.1071 | 12.807 | 4 | 10.00 | 10.00 | 6 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.69656 | 0.844 | 0 | 1.8956 |
| Equality of variance cannot be confirmed | | | | |

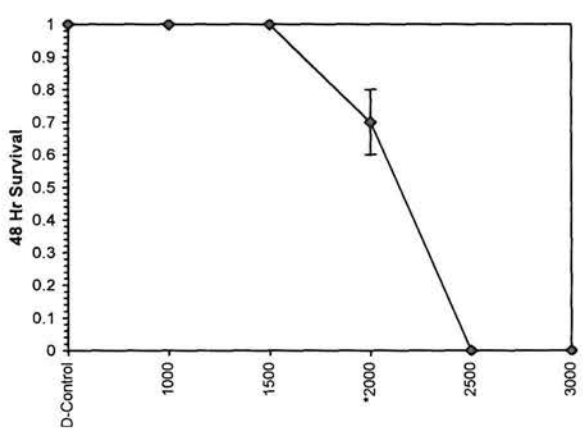
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 1500 | 2000 | 1732.05 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2071.13 | 1965.51 | 2182.43 |
| 5.0% | 2084.8 | 1965.48 | 2211.38 |
| 10.0% | 2097.82 | 1958.07 | 2247.55 |
| 20.0% | 2120.32 | 1907.14 | 2357.34 |
| Auto-0.0% | 2071.13 | 1965.51 | 2182.43 |




### Dose-Response Plot



# 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 #** 5

***Dilution Preparation:***

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2342

***Chemical Analyses:***

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | JP JK | JP JK | JP |
| Control, MHSW | pH (S.U.) | 7.92 | 7.92 | 7.78 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.8 | 7.6 |
| | Conductivity (µmhos/cm) | 297 | | |
| | Alkalinity (mg/L $CaCO_3$) | 59 | | |
| | Hardness (mg/L $CaCO_3$) | 82 | | |
| | Temperature (°C) | 24.4 | 25.2 | 25.7 |
| 1000 mg/L | pH (S.U.) | 7.91 | 7.90 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.8 | 7.7 |
| | Conductivity (µmhos/cm) | 2170 | | |
| | Temperature (°C) | 24.6 | 25.5 | 25.6 |
| 1500 mg/L | pH (S.U.) | 7.92 | 7.91 | 7.84 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.7 | 7.7 |
| | Conductivity (µmhos/cm) | 3040 | | |
| | Temperature (°C) | 24.6 | 25.6 | 25.7 |
| 2000 mg/L | pH (S.U.) | 7.93 | 7.91 | 7.84 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.7 | 7.7 |
| | Conductivity (µmhos/cm) | 4010 | | |
| | Temperature (°C) | 24.6 | 25.7 | 25.8 |
| 2500 mg/L | pH (S.U.) | 7.93 | 7.90 | 7.83 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.7 | 7.8 |
| | Conductivity (µmhos/cm) | 4810 | | |
| | Temperature (°C) | 24.4 | 25.3 | 25.6 |
| 3000 mg/L | pH (S.U.) | 7.94 | 7.91 | |
| | Dissolved oxygen (mg/L) | 8.0 | 7.7 | |
| | Conductivity (µmhos/cm) | 5150 | | |
| | Temperature (°C) | 24.8 | 25.6 | |

JP 01-29-25

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

***Chemical analyses:***

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O 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 | i306641 |

# 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 # 5

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 01-28-25 | *0846 | JP | 1125 | JP | 2B | Yellow | 01-23-25 |
| 24 | 01-29-25 | | | *1405 | JP | | | |
| 48 Termination | 01-30-25 | | | 1134 | JP | | | |

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

*INVALID TEST EXCEEDED ±2-HOUR FOR 24-HOUR CHECK.

NOT INCLUDED IN CONTROL CHART

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 01-21-25 A+B |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 01-27-25 1313 to 01-28-25 0846 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 8.05 |
| | Temperature (°C): 24.8°C |

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

| Hours | Control | | | | 1000 mg/L | | | | 1500 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3[22] | 5 | 5 | 5 |
| 48 Termination | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3 | 5 | 5 | 5 |
| Mean Survival | 100% | | | | 100% | | | | 80% ~~85%~~ 01-30-25 | | | |

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 4[1d] | 4[1d] | 5 | 5 | 0 | 3[2d] | 2[3d] | 0[5d] | 0[5d] | 0[5d] | 0[5d] | 0[5d] |
| 48 Termination | 3[1d] | 4 | 5 | 4[1d] | 0 | 0[3d] | 0[2d] | 0 | 0 | 0 | 0 | 0 |
| Mean Survival | 70% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Spearman-Karber |
| Lower 95% confidence limit (mg NaCl/L) | 1922.8 |
| Upper 95% confidence limit (mg NaCl/L) | 2243.4 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 2052.3 |

Comments:

Test Reviewed by:

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 1/28/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 1/30/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 0.6000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.8000 | 0.8000 | 1.0000 | 1.0000 |
| 2500 | 0.0000 | 0.6000 | 0.4000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

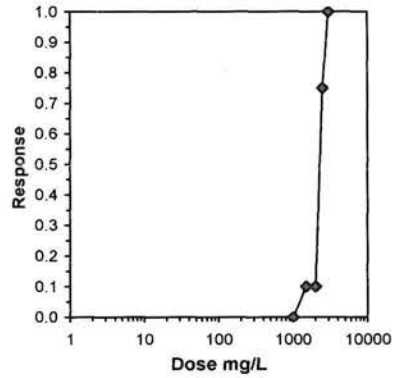
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.9000 | 0.9000 | 1.2305 | 0.8861 | 1.3453 | 18.660 | 4 | 16.00 | 10.00 | 2 | 20 |
| 2000 | 0.9000 | 0.9000 | 1.2262 | 1.1071 | 1.3453 | 11.212 | 4 | 14.00 | 10.00 | 2 | 20 |
| *2500 | 0.2500 | 0.2500 | 0.5055 | 0.2255 | 0.8861 | 65.988 | 4 | 10.00 | 10.00 | 15 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.91642 | 0.868 | -0.2299 | 0.76708 |
| Equality of variance cannot be confirmed | | | | |

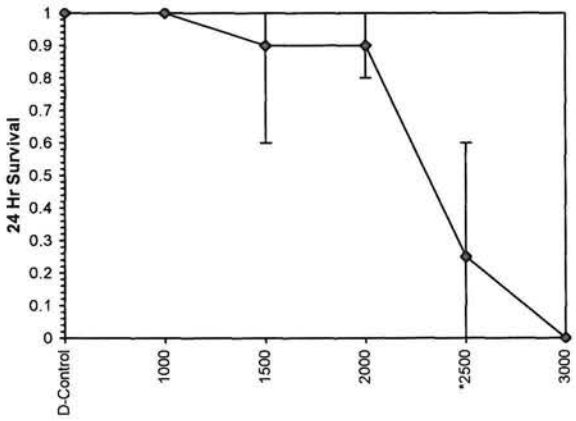
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2000 | 2500 | 2236.07 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2214.89 | 2065.48 | 2375.11 |
| 5.0% | 2265.75 | 2107.68 | 2435.68 |
| 10.0% | 2306.88 | 2196.04 | 2423.3 |
| 20.0% | 2296.23 | 2182.71 | 2415.67 |
| Auto-0.0% | 2214.89 | 2065.48 | 2375.11 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 1/28/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 1/30/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 0.6000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.6000 | 0.8000 | 1.0000 | 0.8000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

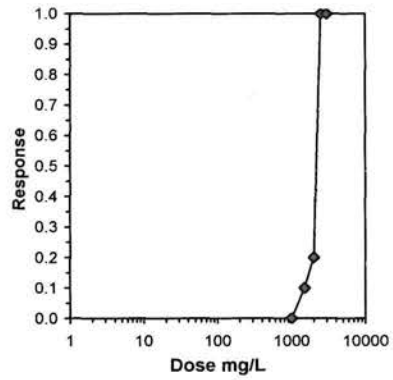
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.9000 | 0.9000 | 1.2305 | 0.8861 | 1.3453 | 18.660 | 4 | 16.00 | 10.00 | 2 | 20 |
| 2000 | 0.8000 | 0.8000 | 1.1114 | 0.8861 | 1.3453 | 16.874 | 4 | 12.00 | 10.00 | 4 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.79345 | 0.844 | -1.1431 | 2.79243 |
| Equality of variance cannot be confirmed | | | | |

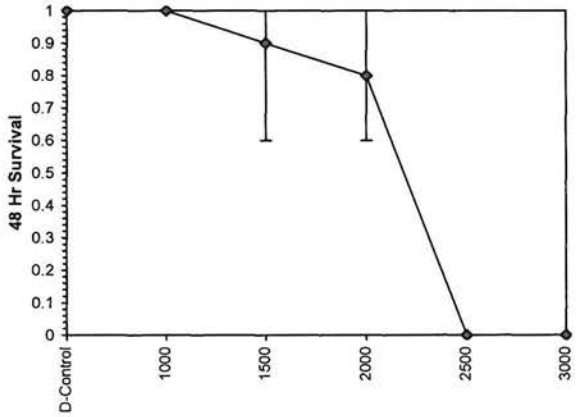
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2000 | 2500 | 2236.07 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2052.34 | 1922.81 | 2190.58 |
| 5.0% | 2101.64 | 1965.07 | 2247.69 |
| 10.0% | 2139.54 | 2011.64 | 2275.56 |
| 20.0% | 2174.56 | 2107.79 | 2243.44 |
| Auto-0.0% | 2052.34 | 1922.81 | 2190.58 |




### Dose-Response Plot



# 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 #** 6

### *Dilution Preparation:*

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2342

### *Chemical Analyses:*

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | NM | AP | AP EW |
| Control, MHSW | pH (S.U.) | 8.05 | 7.78 | 7.94 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.9 | 7.7 |
| | Conductivity (µmhos/cm) | 300 | | |
| | Alkalinity (mg/L $CaCO_3$) | 61 | | |
| | Hardness (mg/L $CaCO_3$) | 90 | | |
| | Temperature (°C) | 25.4 | 25.2 | 24.6 |
| 1000 mg/L | pH (S.U.) | 7.99 | 7.78 | 7.87 |
| | Dissolved oxygen (mg/L) | 7.9 | 7.9 | 7.7 |
| | Conductivity (µmhos/cm) | 2100 | | |
| | Temperature (°C) | 25.4 | 25.4 | 25.0 |
| 1500 mg/L | pH (S.U.) | 7.99 | 7.77 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.8 | 7.7 |
| | Conductivity (µmhos/cm) | 2910 | | |
| | Temperature (°C) | 25.3 | 25.1 | 24.8 |
| 2000 mg/L | pH (S.U.) | 7.96 | 7.76 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.8 | 7.7 |
| | Conductivity (µmhos/cm) | 3760 | | |
| | Temperature (°C) | 25.3 | 25.0 | 24.8 |
| 2500 mg/L | pH (S.U.) | 7.95 | 7.76 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.8 | 7.6 |
| | Conductivity (µmhos/cm) | 4570 | | |
| | Temperature (°C) | 25.6 | 25.0 | 24.7 |
| 3000 mg/L | pH (S.U.) | 7.94 | 7.75 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.8 | 7.7 |
| | Conductivity (µmhos/cm) | 5440 | | |
| | Temperature (°C) | 25.2 | 25.0 | 24.8 |

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

### *Chemical analyses:*

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O 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 | 130664 |

# 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 # 6

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 02-04-25 | *0906 | JP | 1123 | JP | 2C | Yellow | 02-02-25A |
| 24 | 02-05-25 | | | 1125 | JP | | | |
| 48 Termination | 02-06-25 | | | 1117 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 01-28-25 A+B |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 02-03-25 1321 to 02-04-25 0906 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 8.17 |
| | Temperature (°C): 24.7 |

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

| Hours | Control | | | | 1000 mg/L | | | | 1500 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 1d | 5 | 5 | 5 |
| 48 Termination | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 5 | 5 |
| Mean Survival | 100% | | | | 100% | | | | 95% | | | |

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 4 1d | 5 | 5 | 5 | 5 | 1 4d | 3 2d | 2 3d | 0 5d | 2 3d | 1 4d | 0 5d |
| 48 Termination | 4 | 5 | 4 1d | 5 | 0 5d | 0 1d | 0 3d | 0 2d | 0 | 0 2d | 0 1d | 0 |
| Mean Survival | 90% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | | Comments: |
|---|---|---|
| Method | Spearman-Karber | 2041.6 |
| Lower 95% confidence limit (mg NaCl/L) | ~~2142.2~~ 2041.6 | |
| Upper 95% confidence limit (mg NaCl/L) | 2247.8 | |
| 48-hour $LC_{50}$ (mg NaCl/L) | 2142.2 | |

Test Reviewed by: [illegible]

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 0.8000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.8000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 1.0000 | 0.2000 | 0.6000 | 0.4000 |
| 3000 | 0.0000 | 0.4000 | 0.2000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.9500 | 0.9500 | 1.2857 | 1.1071 | 1.3453 | 9.261 | 4 | 16.00 | 10.00 | 1 | 20 |
| 2000 | 0.9500 | 0.9500 | 1.2857 | 1.1071 | 1.3453 | 9.261 | 4 | 16.00 | 10.00 | 1 | 20 |
| 2500 | 0.5500 | 0.5500 | 0.8449 | 0.4636 | 1.3453 | 44.446 | 4 | 12.00 | 10.00 | 9 | 20 |
| *3000 | 0.1500 | 0.1500 | 0.3998 | 0.2255 | 0.6847 | 55.174 | 4 | 10.00 | 10.00 | 17 | 20 |

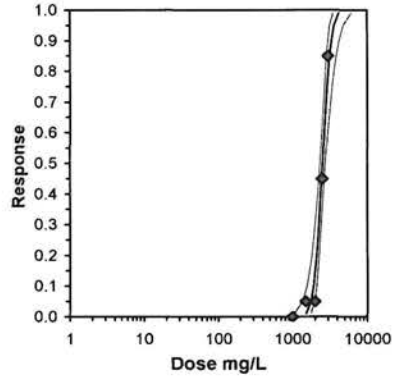
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.85387 | 0.884 | 0.67679 | 3.1984 |
| Equality of variance cannot be confirmed | | | | |

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

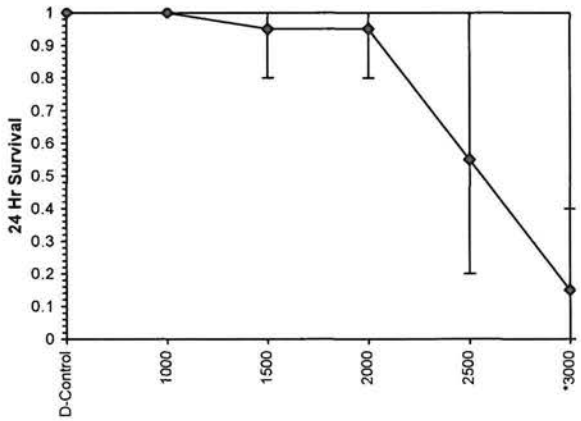
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 10.5758 | 2.22711 | 6.21064 | 14.9409 | 0 | 6.09096 | 7.81472 | 0.10727 | 3.40197 | 0.09456 | 4 |
| Intercept | -30.978 | 7.5497 | -45.776 | -16.181 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1520.55 | 1072.71 | 1772.17 |
| EC05 | 3.355 | 1763.76 | 1373.22 | 1979.65 |
| EC10 | 3.718 | 1908.93 | 1562.96 | 2104.72 |
| EC15 | 3.964 | 2013.58 | 1702.64 | 2197.32 |
| EC20 | 4.158 | 2100.82 | 1819.55 | 2277.49 |
| EC25 | 4.326 | 2178.67 | 1922.99 | 2352.57 |
| EC40 | 4.747 | 2387.89 | 2185.71 | 2581.8 |
| EC50 | 5.000 | 2523.3 | 2335.1 | 2760.32 |
| EC60 | 5.253 | 2666.39 | 2473.51 | 2976.46 |
| EC75 | 5.674 | 2922.44 | 2686.58 | 3418.31 |
| EC80 | 5.842 | 3030.74 | 2768.6 | 3621.15 |
| EC85 | 6.036 | 3162.06 | 2864.08 | 3877.3 |
| EC90 | 6.282 | 3335.39 | 2985.26 | 4230.62 |
| EC95 | 6.645 | 3609.94 | 3169.32 | 4822.07 |
| EC99 | 7.326 | 4187.34 | 3535.85 | 6180.85 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 0.8000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.8000 | 1.0000 | 0.8000 | 1.0000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

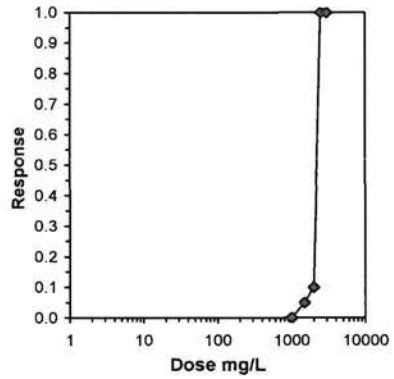
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.9500 | 0.9500 | 1.2857 | 1.1071 | 1.3453 | 9.261 | 4 | 16.00 | 10.00 | 1 | 20 |
| 2000 | 0.9000 | 0.9000 | 1.2262 | 1.1071 | 1.3453 | 11.212 | 4 | 14.00 | 10.00 | 2 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.87117 | 0.844 | -0.717 | 0.5231 |
| Equality of variance cannot be confirmed | | | | |

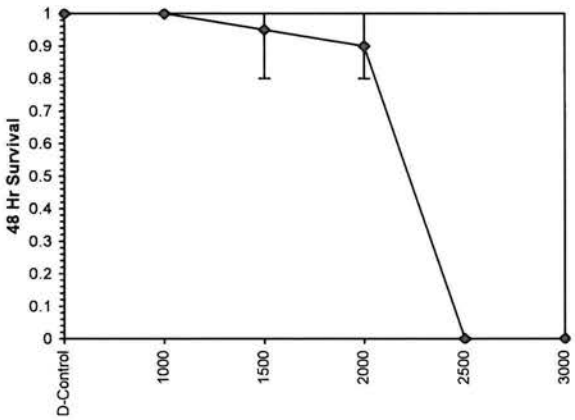
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2000 | 2500 | 2236.07 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2142.23 | 2041.59 | 2247.83 |
| 5.0% | 2191.7 | 2103.46 | 2283.63 |
| 10.0% | 2208.52 | 2168.08 | 2249.71 |
| 20.0% | 2208.52 | 2168.08 | 2249.71 |
| Auto-0.0% | 2142.23 | 2041.59 | 2247.83 |




### Dose-Response Plot



# 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 #** 1

***Dilution Preparation:***

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2356

***Chemical Analyses:***

| | | Hours | | |
|---|---|---|---|---|
| | | 0 | 24 | 48 |
| Concentration | Analyst | JP | JP EM | JP |
| Control, MHSW | pH (S.U.) | 7.88 | 7.92 | 7.75 |
| | Dissolved oxygen (mg/L) | 7.8 | 8.1 | 7.7 |
| | Conductivity (µmhos/cm) | 306 | | |
| | Alkalinity (mg/L $CaCO_3$) | 63 | | |
| | Hardness (mg/L $CaCO_3$) | 86 | | |
| | Temperature (°C) | 25.6 | 25.5 | 25.7 |
| 1000 mg/L | pH (S.U.) | 7.95 | 7.91 | 7.83 |
| | Dissolved oxygen (mg/L) | 7.8 | 8.1 | 7.9 |
| | Conductivity (µmhos/cm) | 2130 | | |
| | Temperature (°C) | 25.8 | 25.0 | 24.8 |
| 1500 mg/L | pH (S.U.) | 7.95 | 7.91 | 7.84 |
| | Dissolved oxygen (mg/L) | 7.7 | 8.1 | 7.9 |
| | Conductivity (µmhos/cm) | 3060 | | |
| | Temperature (°C) | 24.2 | 25.7 | 25.4 |
| 2000 mg/L | pH (S.U.) | 7.94 | 7.90 | 7.83 |
| | Dissolved oxygen (mg/L) | 7.7 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 3940 | | |
| | Temperature (°C) | 24.1 | 25.9 | 25.6 |
| 2500 mg/L | pH (S.U.) | 7.93 | 7.90 | 7.84 |
| | Dissolved oxygen (mg/L) | 7.7 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 4720 | | |
| | Temperature (°C) | 25.6 | 25.6 | 25.6 |
| 3000 mg/L | pH (S.U.) | 7.93 | 7.91 | 7.83 |
| | Dissolved oxygen (mg/L) | 7.7 | 8.2 | 8.1 |
| | Conductivity (µmhos/cm) | 5720 | | |
| | Temperature (°C) | 25.7 | 25.3 | 25.5 |

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

***Chemical analyses:***

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O 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 | 130662 |

# 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 # 7

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 02-11-25 | *~~0900~~ 0810 | 02-11-25 JP | ~~0945~~ 1025 | 02-11-25 JP | 1C | BIOAK | 02-08-25 |
| 24 | 02-12-25 | | | 1010 | JP | | | |
| 48 Termination | 02-13-25 | | | 1006 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 02-04-25 |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 02-10-25 1202 to 02-11-25 0904 * |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.80 |
| | Temperature (°C): 24.1°C |

* INVALID TEST ORGANISMS DID NOT HAVE MINIMUM OF 2-HOURS FOR FEEDING & NOT INCLUDED IN CONTROL CHAR

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

| Hours | Control | | | | 1000 mg/L | | | | 1500 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 48 Termination | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | $3^{2d}$ | $1^{4d}$ |
| Mean Survival | 100% | | | | 100% | | | | 70% | 80% JP 02-13-25 | | |

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | $4^{1d}$ | 5 | 5 | 5 | $0^{5d}$ | 5 | $2^{3d}$ | 5 | $0^{5d}$ | $0^{5d}$ | $0^{5d}$ | $2^{3d}$ |
| 48 Termination | $1^{3d}$ | $4^{1d}$ | $2^{3d}$ | $4^{1d}$ | 0 | $0^{5d}$ | $0^{2d}$ | $0^{5d}$ | 0 | 0 | 0 | $0^{2d}$ |
| Mean Survival | 55% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Probit |
| Lower 95% confidence limit (mg NaCl/L) | 1652.1 |
| Upper 95% confidence limit (mg NaCl/L) | 1967.7 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1815.8 |

Comments:

Test Reviewed by: JP

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 2/11/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 2/13/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.8000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 0.0000 | 1.0000 | 0.4000 | 1.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.4000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 0.9500 | 0.9500 | 1.2857 | 1.1071 | 1.3453 | 9.261 | 4 | 16.00 | 10.00 | 1 | 20 |
| 2500 | 0.6000 | 0.6000 | 0.9002 | 0.2255 | 1.3453 | 60.771 | 4 | 14.00 | 10.00 | 8 | 20 |
| *3000 | 0.1000 | 0.1000 | 0.3403 | 0.2255 | 0.6847 | 67.468 | 4 | 10.00 | 10.00 | 18 | 20 |

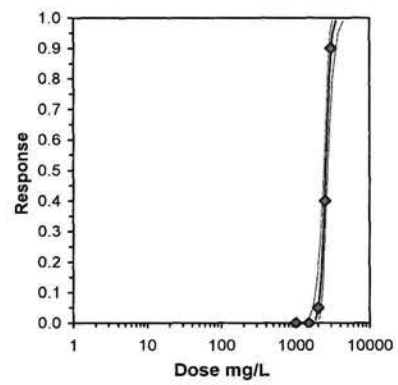
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.79853 | 0.884 | -0.4977 | 4.07734 |
| Equality of variance cannot be confirmed | | | | |

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

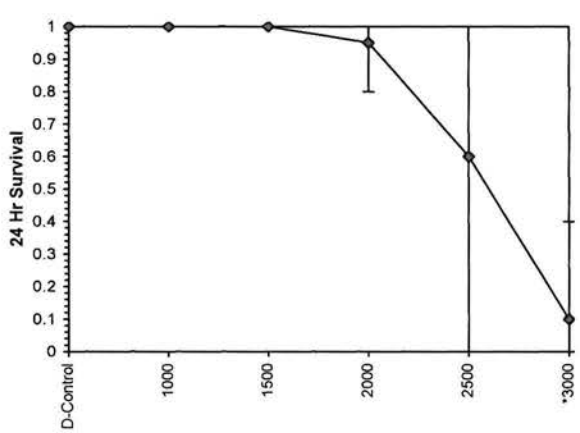
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 16.8501 | 3.52916 | 9.93294 | 23.7673 | 0 | 0.28669 | 7.81472 | 0.96251 | 3.40692 | 0.05935 | 3 |
| Intercept | -52.407 | 12.0284 | -75.983 | -28.831 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1857.2 | 1469.94 | 2061.1 |
| EC05 | 3.355 | 2038.46 | 1714.52 | 2210.75 |
| EC10 | 3.718 | 2142.22 | 1858.56 | 2298.09 |
| EC15 | 3.964 | 2215.19 | 1960.57 | 2361.3 |
| EC20 | 4.158 | 2274.95 | 2043.87 | 2414.85 |
| EC25 | 4.326 | 2327.51 | 2116.34 | 2463.86 |
| EC40 | 4.747 | 2465.38 | 2298.18 | 2605.76 |
| EC50 | 5.000 | 2552.23 | 2402.25 | 2709.35 |
| EC60 | 5.253 | 2642.14 | 2499.11 | 2830.51 |
| EC75 | 5.674 | 2798.65 | 2644.78 | 3071.7 |
| EC80 | 5.842 | 2863.3 | 2698.9 | 3180.09 |
| EC85 | 6.036 | 2940.55 | 2760.51 | 3314.72 |
| EC90 | 6.282 | 3040.71 | 2836.78 | 3496.23 |
| EC95 | 6.645 | 3195.48 | 2949.21 | 3789.5 |
| EC99 | 7.326 | 3507.37 | 3163.68 | 4419.55 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 2/11/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 2/13/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 0.6000 | 0.2000 |
| 2000 | 0.2000 | 0.8000 | 0.4000 | 0.8000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.7000 | 0.7000 | 1.0101 | 0.4636 | 1.3453 | 41.952 | 4 | 14.00 | 10.00 | 6 | 20 |
| *2000 | 0.5500 | 0.5500 | 0.8407 | 0.4636 | 1.1071 | 38.145 | 4 | 10.00 | 10.00 | 9 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

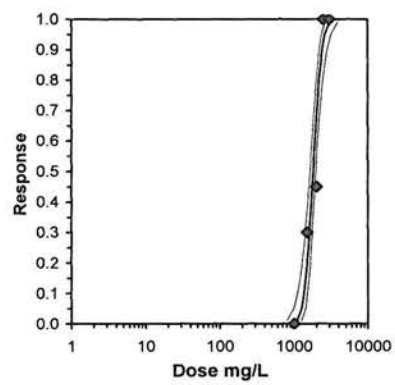
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.87767 | 0.844 | -0.6202 | 0.83655 |
| Equality of variance cannot be confirmed | | | | |

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

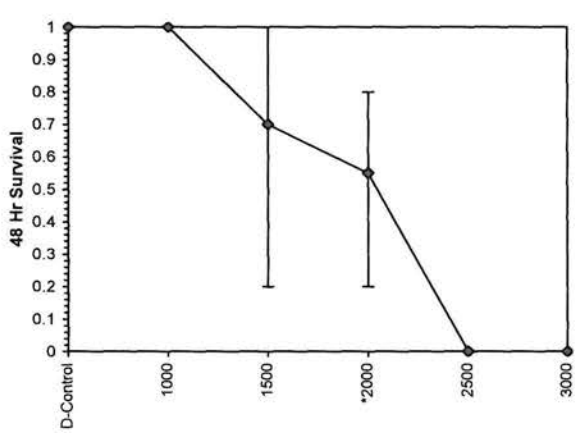
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 10.4061 | 1.82025 | 6.83843 | 13.9738 | 0 | 7.66789 | 7.81472 | 0.0534 | 3.25907 | 0.0961 | 6 |
| Intercept | -28.914 | 5.97714 | -40.629 | -17.199 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1085.21 | 794.319 | 1274.49 |
| EC05 | 3.355 | 1261.84 | 994.085 | 1433.29 |
| EC10 | 3.718 | 1367.46 | 1118.68 | 1528.19 |
| EC15 | 3.964 | 1443.68 | 1210.23 | 1597.35 |
| EC20 | 4.158 | 1507.27 | 1287.21 | 1655.94 |
| EC25 | 4.326 | 1564.06 | 1356.06 | 1709.29 |
| EC40 | 4.747 | 1716.81 | 1538.77 | 1860.57 |
| EC50 | 5.000 | 1815.81 | 1652.08 | 1967.73 |
| EC60 | 5.253 | 1920.5 | 1764.77 | 2091.64 |
| EC75 | 5.674 | 2108.07 | 1946 | 2342.9 |
| EC80 | 5.842 | 2187.49 | 2015.82 | 2459.49 |
| EC85 | 6.036 | 2283.85 | 2096.4 | 2607.66 |
| EC90 | 6.282 | 2411.14 | 2197.6 | 2812.96 |
| EC95 | 6.645 | 2612.97 | 2349.57 | 3156.81 |
| EC99 | 7.326 | 3038.26 | 2649.34 | 3940.26 |




### Dose-Response Plot



# 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 #** 8

***Dilution Preparation:***

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2356

***Chemical Analyses:***

| | | Hours | | |
|---|---|---|---|---|
| | | 0 | 24 | 48 |
| Concentration | Analyst | JP | JP | JP |
| Control, MHSW | pH (S.U.) | 7.93 | 7.99 | 7.90 |
| | Dissolved oxygen (mg/L) | 8.0 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 317 | | |
| | Alkalinity (mg/L $CaCO_3$) | 62 | | |
| | Hardness (mg/L $CaCO_3$) | 80 | | |
| | Temperature (°C) | 24.5 | 25.4 | 25.5 |
| 1000 mg/L | pH (S.U.) | 7.95 | 7.94 | 7.91 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 2130 | | |
| | Temperature (°C) | 24.5 | 24.5 | 25.4 |
| 1500 mg/L | pH (S.U.) | 7.98 | 7.94 | 7.91 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 3090 | | |
| | Temperature (°C) | 24.2 | 24.7 | 25.6 |
| 2000 mg/L | pH (S.U.) | 7.97 | 7.93 | 7.90 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 3960 | | |
| | Temperature (°C) | 24.8 | 24.7 | 25.2 |
| 2500 mg/L | pH (S.U.) | 7.96 | 7.95 | 7.89 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 4840 | | |
| | Temperature (°C) | 24.6 | 24.1 | 25.5 |
| 3000 mg/L | pH (S.U.) | 7.95 | 7.96 | |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | |
| | Conductivity (µmhos/cm) | 5700 | | |
| | Temperature (°C) | 24.5 | 25.0 | JP 02-26-25 |

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measuremer only. Temperatures performed at the time of test initiation or termination by the analyst performing the toxicity test. Alkalinity and hardness performed by the analysts identified on tl 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 | 1306046 |

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

***Ceriodaphnia dubia*** **Sodium Chloride Acute Reference Toxicant Test**

CdNaClAC # 3

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 02-25-25 | *0846 | JP | 1100 | JP | 1C | Rez | 02-19-25 |
| 24 | 02-26-25 | | | 1056 | JP | | | |
| 48 Termination | 02-27-25 | | | 1100 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 02-18-25 (A) ~~A+B~~ broods K |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 02-24-25 1152 to<br>02-25-25 0846 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.80 |
| | Temperature (°C): 24.2°C |

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

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

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 4 1d | 5 | 5 | 5 | 5 | 4 1d | 3 2d | 4 1d | 0 5d | 0 5d | 0 5d | 0 5d |
| 48 Termination | 3 1d | 0 | 3 2d | 1 4d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean Survival | 35% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Spearman-Karber |
| Lower 95% confidence limit (mg NaCl/L) | 1793.6 |
| Upper 95% confidence limit (mg NaCl/L) | 2000.1 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1894.0 |

Comments:

Test Reviewed by: [illegible]

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 2/25/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 2/27/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.8000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 1.0000 | 0.8000 | 0.6000 | 0.8000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

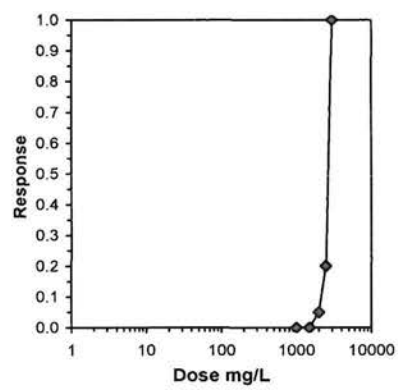
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 0.9500 | 0.9500 | 1.2857 | 1.1071 | 1.3453 | 9.261 | 4 | 16.00 | 10.00 | 1 | 20 |
| 2500 | 0.8000 | 0.8000 | 1.1114 | 0.8861 | 1.3453 | 16.874 | 4 | 12.00 | 10.00 | 4 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.70701 | 0.868 | -0.3159 | 4.3442 |
| Equality of variance cannot be confirmed | | | | |

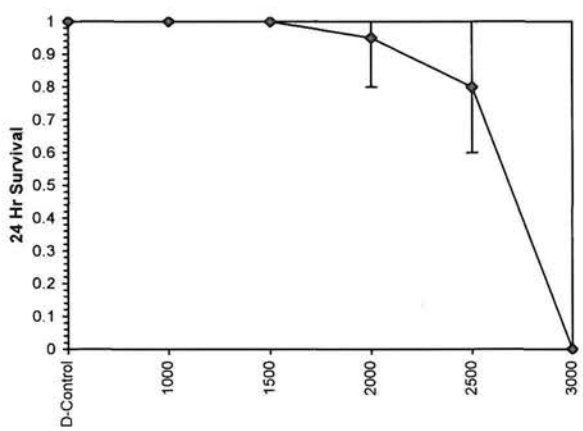
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2500 | 3000 | 2738.61 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2596.42 | 2484.69 | 2713.18 |
| 5.0% | 2635.08 | 2526.7 | 2748.11 |
| 10.0% | 2655.91 | 2531.02 | 2786.97 |
| 20.0% | 2676.91 | 2609.56 | 2745.99 |
| Auto-0.0% | 2596.42 | 2484.69 | 2713.18 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 2/25/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 2/27/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.6000 | 0.0000 | 0.6000 | 0.2000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

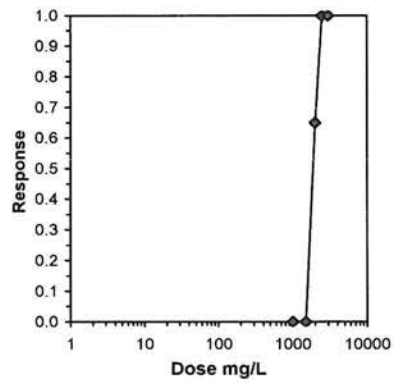
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2000 | 0.3500 | 0.3500 | 0.6153 | 0.2255 | 0.8861 | 53.207 | 4 | 10.00 | 10.00 | 13 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.69562 | 0.844 | -0.559 | 3.74151 |
| Equality of variance cannot be confirmed | | | | |

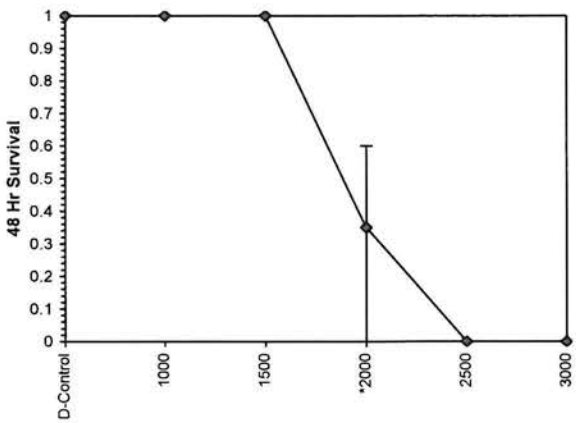
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 1500 | 2000 | 1732.05 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 1894.02 | 1793.59 | 2000.07 |
| 5.0% | 1889.87 | 1779.46 | 2007.13 |
| 10.0% | 1885.84 | 1764.37 | 2015.68 |
| 20.0% | 1878.39 | 1729.31 | 2040.32 |
| Auto-0.0% | 1894.02 | 1793.59 | 2000.07 |




### Dose-Response Plot



# 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 # 9

### *Dilution Preparation:*

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

Stock solution INSS #: 2356

### *Chemical Analyses:*

| Concentration | Analyst | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| Control, MHSW | pH (S.U.) | 7.96 | 8.05 | 7.98 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 313 | | |
| | Alkalinity (mg/L $CaCO_3$) | 60 | | |
| | Hardness (mg/L $CaCO_3$) | 84 | | |
| | Temperature (°C) | 25.1 | ~~24.9~~ 25.5 | 25.1 |
| 1000 mg/L | pH (S.U.) | 7.96 | 7.98 | 7.97 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 2060 | | |
| | Temperature (°C) | 25.3 | ~~24.4~~ 25.5 | 25.0 |
| 1500 mg/L | pH (S.U.) | 7.95 | 7.98 | 7.96 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 2920 | | |
| | Temperature (°C) | 25.2 | ~~24.4~~ 25.3 | 25.0 |
| 2000 mg/L | pH (S.U.) | 7.94 | 7.97 | 7.93 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 3760 | | |
| | Temperature (°C) | 25.4 | 25.3 | 25.3 |
| 2500 mg/L | pH (S.U.) | 7.94 | 7.96 | 7.94 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | 8.1 |
| | Conductivity (µmhos/cm) | 4570 | | |
| | Temperature (°C) | 25.3 | 25.5 | 25.1 |
| 3000 mg/L | pH (S.U.) | 7.94 | 7.94 | 7.94 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 5430 | | |
| | Temperature (°C) | 25.1 | 25.6 | 25.3 |

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

### *Chemical analyses:*

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O 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 | ~~130664~~ 130664 |

# 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 # 9

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 03-04-25 | * 0800 | K | 1135 | JP | 2C | Green | 02-26-25 B |
| 24 | 03-05-25 | | | 1140 | JP | | | |
| 48 Termination | 03-06-25 | | | 1125 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 02-25-25 D - JIM CULTURE |
| 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): 24.3 C |

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

| Hours | Control | | | | 1000 mg/L | | | | 1500 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 1d |
| 48 Termination | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 |
| Mean Survival | 100% | | | | 100% | | | | 95% | | | |

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 4 1d | 2 3d | 3 2d | 5 | 4 1d | 4 1d | 4 1d | 5 | 2 3d | 0 5d | 1 4d | 3 2d |
| 48 Termination | 1 3d | 2 | 3 | 2 3d | 0 4d | 0 4d | 0 4d | 0 5d | 0 2d | 0 | 0 1d | 0 3d |
| Mean Survival | 40% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Probit |
| Lower 95% confidence limit (mg NaCl/L) | 1770.2 |
| Upper 95% confidence limit (mg NaCl/L) | 2024.6 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1901.9 |

Comments:

Test Reviewed by: [signature]

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 3/04/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 3/06/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 0.8000 |
| 2000 | 0.8000 | 0.4000 | 0.6000 | 1.0000 |
| 2500 | 0.8000 | 0.8000 | 0.8000 | 1.0000 |
| 3000 | 0.4000 | 0.0000 | 0.2000 | 0.6000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.9500 | 0.9500 | 1.2857 | 1.1071 | 1.3453 | 9.261 | 4 | 16.00 | 10.00 | 1 | 20 |
| 2000 | 0.7000 | 0.7000 | 1.0058 | 0.6847 | 1.3453 | 28.293 | 4 | 12.00 | 10.00 | 6 | 20 |
| 2500 | 0.8500 | 0.8500 | 1.1667 | 1.1071 | 1.3453 | 10.206 | 4 | 12.00 | 10.00 | 3 | 20 |
| *3000 | 0.3000 | 0.3000 | 0.5650 | 0.2255 | 0.8861 | 50.368 | 4 | 10.00 | 10.00 | 14 | 20 |

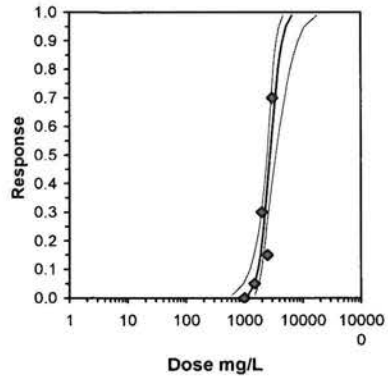
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.92802 | 0.884 | 7.5E-15 | 1.19704 |
| Equality of variance cannot be confirmed | | | | |

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

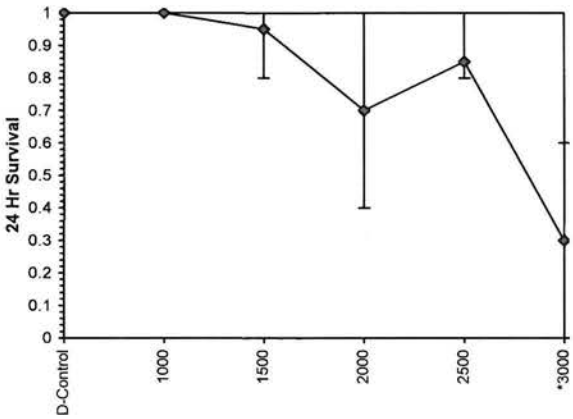
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 6.13845 | 1.49669 | 3.20494 | 9.07197 | 0 | 7.61656 | 7.81472 | 0.05464 | 3.44477 | 0.16291 | 3 |
| Intercept | -16.146 | 5.04455 | -26.033 | -6.2583 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1163.58 | 602.211 | 1487.59 |
| EC05 | 3.355 | 1502.5 | 972.245 | 1787.38 |
| EC10 | 3.718 | 1721.87 | 1247.82 | 1982.64 |
| EC15 | 3.964 | 1887.69 | 1468.52 | 2138.05 |
| EC20 | 4.158 | 2030.8 | 1661.29 | 2284.07 |
| EC25 | 4.326 | 2162.19 | 1833.69 | 2434.45 |
| EC40 | 4.747 | 2532.22 | 2249.38 | 2988.76 |
| EC50 | 5.000 | 2784.67 | 2469.97 | 3482.07 |
| EC60 | 5.253 | 3062.29 | 2678.59 | 4107.7 |
| EC75 | 5.674 | 3586.36 | 3025.75 | 5476.43 |
| EC80 | 5.842 | 3818.39 | 3168.49 | 6152.47 |
| EC85 | 6.036 | 4107.87 | 3340.32 | 7052.99 |
| EC90 | 6.282 | 4503.48 | 3566.34 | 8383.77 |
| EC95 | 6.645 | 5160.99 | 3924.63 | 10845.9 |
| EC99 | 7.326 | 6664.28 | 4685.52 | 17622.5 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 3/04/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 3/06/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 0.8000 |
| 2000 | 0.2000 | 0.4000 | 0.6000 | 0.4000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.9500 | 0.9500 | 1.2857 | 1.1071 | 1.3453 | 9.261 | 4 | 16.00 | 10.00 | 1 | 20 |
| *2000 | 0.4000 | 0.4000 | 0.6798 | 0.4636 | 0.8861 | 25.383 | 4 | 10.00 | 10.00 | 12 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

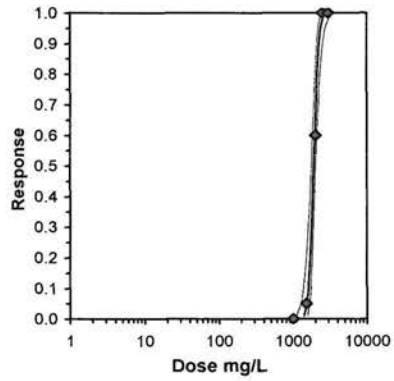
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.77511 | 0.844 | -0.5901 | 2.80133 |
| Equality of variance cannot be confirmed | | | | |

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

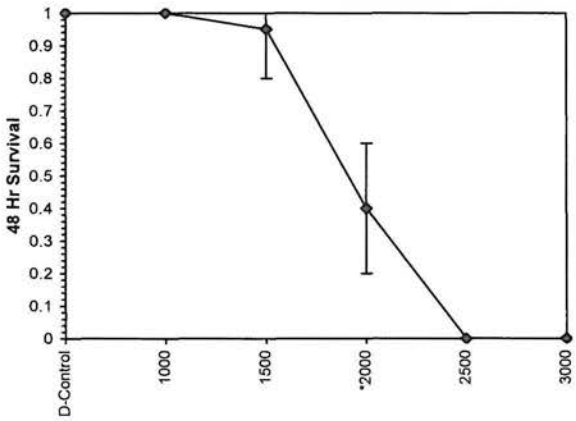
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 17.6691 | 3.65602 | 10.5033 | 24.8349 | 0 | 0.74139 | 7.81472 | 0.86343 | 3.27918 | 0.0566 | 4 |
| Intercept | -52.94 | 12.028 | -76.515 | -29.365 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1404.48 | 1109.27 | 1563.8 |
| EC05 | 3.355 | 1534.92 | 1282.39 | 1673.1 |
| EC10 | 3.718 | 1609.33 | 1383.68 | 1736.7 |
| EC15 | 3.964 | 1661.57 | 1455.25 | 1782.5 |
| EC20 | 4.158 | 1704.29 | 1513.68 | 1821.06 |
| EC25 | 4.326 | 1741.82 | 1564.64 | 1856.03 |
| EC40 | 4.747 | 1840.09 | 1694.07 | 1954.89 |
| EC50 | 5.000 | 1901.85 | 1770.23 | 2024.64 |
| EC60 | 5.253 | 1965.69 | 1843.03 | 2104.58 |
| EC75 | 5.674 | 2076.59 | 1954.64 | 2263.01 |
| EC80 | 5.842 | 2122.31 | 1996.03 | 2334.68 |
| EC85 | 6.036 | 2176.88 | 2042.78 | 2424.17 |
| EC90 | 6.282 | 2247.54 | 2100.05 | 2545.44 |
| EC95 | 6.645 | 2356.51 | 2183.33 | 2742.15 |
| EC99 | 7.326 | 2575.36 | 2339.57 | 3165.19 |




### Dose-Response Plot



# 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 #** .10

***Dilution Preparation:***

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2368

***Chemical Analyses:***

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | JP SB | JP EM | JP |
| Control, MHSW | pH (S.U.) | 7.86 | 7.90 | 7.89 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.1 | 8.1 |
| | Conductivity (µmhos/cm) | 295 | | |
| | Alkalinity (mg/L $CaCO_3$) | 62 | | |
| | Hardness (mg/L $CaCO_3$) | 84 | | |
| | Temperature (°C) | 25.0 | 25.6 | 25.3 |
| 1000 mg/L | pH (S.U.) | 8.01 | 7.92 | 7.89 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.1 | 8.2 |
| | Conductivity (µmhos/cm) | 2090 | | |
| | Temperature (°C) | 25.3 | 25.7 | 25.4 |
| 1500 mg/L | pH (S.U.) | 8.04 | 7.93 | 7.90 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 8.2 |
| | Conductivity (µmhos/cm) | 2900 | | |
| | Temperature (°C) | 25.1 | 25.6 | 25.3 |
| 2000 mg/L | pH (S.U.) | 8.04 | 7.93 | 7.90 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 8.2 |
| | Conductivity (µmhos/cm) | 3840 | | |
| | Temperature (°C) | 25.2 | 25.7 | 25.4 |
| 2500 mg/L | pH (S.U.) | 8.03 | 7.92 | 7.91 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 8.2 |
| | Conductivity (µmhos/cm) | 4660 | | |
| | Temperature (°C) | 25.2 | 25.6 | 25.2 |
| 3000 mg/L | pH (S.U.) | 8.03 | 7.93 | |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | |
| | Conductivity (µmhos/cm) | 5510 | | |
| | Temperature (°C) | 25.4 | 25.4 | JP 03-12-25 |

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

***Chemical analyses:***

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O 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 | 170664 |

# 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 # 10

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 03-11-25 | *0911 | JP | 1130 | JP | 1B | brve | 03-062 5A |
| 24 | 03-12-25 | | | 1139 | JP | | | |
| 48 Termination | 03-13-25 | | | 1129 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 03-24-25 B |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 03-10-25 1313 to 03-11-25 0911 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.85 |
| | Temperature (°C): 24.5°C |

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

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

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 1 sd | 2 sd | 1 sd | 1 sd | 0 sd | 0 sd | 0 sd | 0 sd |
| 48 Termination | 4 ud | 4 ud | 4 ud | 4 ud | 0 ud | 0 ud | 0 ud | 0 d | 0 | 0 | 0 | 0 |
| Mean Survival | 80% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Spearman Karber |
| Lower 95% confidence limit (mg NaCl/L) | 2029.8 |
| Upper 95% confidence limit (mg NaCl/L) | 2224.0 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 2124.7 |

Comments:

Test Reviewed by: JP

ETS
Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 3/11/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 3/13/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 0.2000 | 0.4000 | 0.2000 | 0.2000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Rank Sum | 1-Tailed Critical | Number Resp | Total Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2500 | 0.2500 | 0.2500 | 0.5189 | 0.4636 | 0.6847 | 21.301 | 4 | 10.00 | 10.00 | 15 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

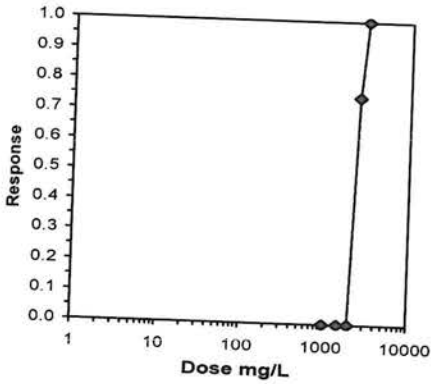
**Auxiliary Tests**

| | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.5089 | 0.868 | 2.79623 | 11.6732 |
| Equality of variance cannot be confirmed | | | | |

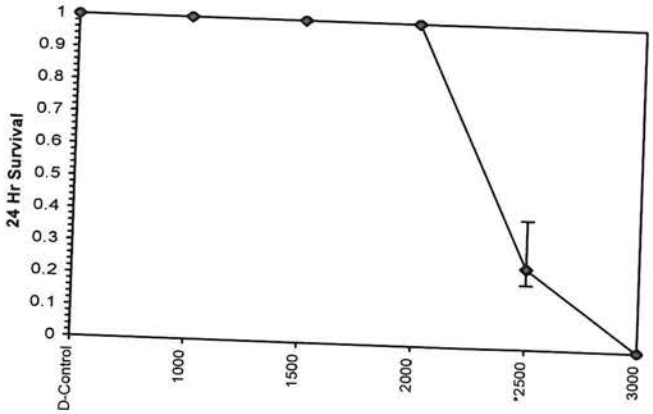
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2000 | 2500 | 2236.07 | |
| Treatments vs D-Control | | | | |

**Trimmed Spearman-Karber**

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2352.32 | 2261.76 | 2446.51 |
| 5.0% | 2343.17 | 2244.68 | 2445.98 |
| 10.0% | 2334.93 | 2229.9 | 2444.9 |
| 20.0% | 2322.88 | 2211.45 | 2439.93 |
| Auto-0.0% | 2352.32 | 2261.76 | 2446.51 |




**Dose-Response Plot**



ETS
Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 3/11/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 3/13/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.8000 | 0.8000 | 0.8000 | 0.8000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Rank Sum | 1-Tailed Critical | Number Resp | Total Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2000 | 0.8000 | 0.8000 | 1.1071 | 1.1071 | 1.1071 | 0.000 | 4 | 10.00 | 10.00 | 4 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

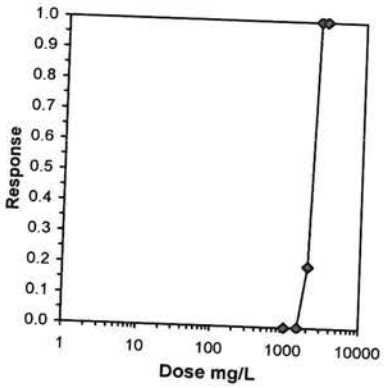
**Auxiliary Tests**

| | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 1 | 0.844 | | |
| Equality of variance cannot be confirmed | | | | |

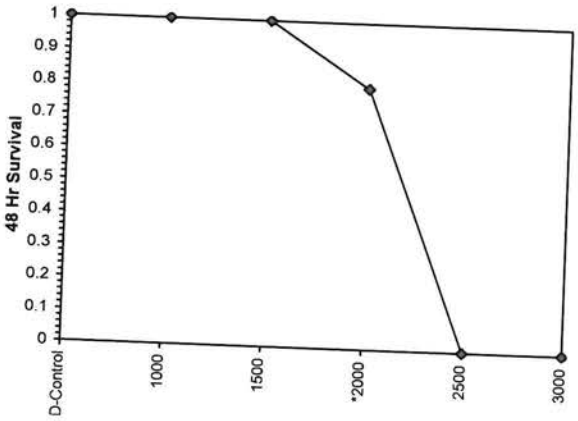
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 1500 | 2000 | 1732.05 | |
| Treatments vs D-Control | | | | |

**Trimmed Spearman-Karber**

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2124.71 | 2029.82 | 2224.04 |
| 5.0% | 2143.27 | 2033.72 | 2258.72 |
| 10.0% | 2158.86 | 2023.47 | 2303.3 |
| 20.0% | 2174.56 | 2107.79 | 2243.44 |
| Auto-0.0% | 2124.71 | 2029.82 | 2224.04 |




**Dose-Response Plot**



ETC

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

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



## *Ceriodaphnia dubia* Sodium Chloride Acute Reference Toxicant Test

CdNaClAC # 11

### *Dilution Preparation:*

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| 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 stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2368

### *Chemical Analyses:*

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | AP K | AP EM | AP K |
| Control, MHSW | pH (S.U.) | 7.75 | 7.91 | 7.89 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.2 | 8.1 |
| | Conductivity (µmhos/cm) | 302 | | |
| | Alkalinity (mg/L $CaCO_3$) | 59 | | |
| | Hardness (mg/L $CaCO_3$) | 86 | | |
| | Temperature (°C) | 25.5 | 25.4 | 25.5 |
| 1000 mg/L | pH (S.U.) | 7.76 | 7.90 | 7.89 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.2 | 8.1 |
| | Conductivity (µmhos/cm) | 2050 | | |
| | Temperature (°C) | 25.5 | 25.3 | 25.6 |
| 1500 mg/L | pH (S.U.) | 7.77 | 7.90 | 7.89 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.2 | 8.1 |
| | Conductivity (µmhos/cm) | 2970 | | |
| | Temperature (°C) | 25.4 | 25.5 | 25.6 |
| 2000 mg/L | pH (S.U.) | 7.78 | 7.89 | 7.90 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.2 | 8.1 |
| | Conductivity (µmhos/cm) | 3840 | | |
| | Temperature (°C) | 25.4 | 25.7 | 25.7 |
| 2500 mg/L | pH (S.U.) | 7.81 | 7.90 | 7.90 |
| | Dissolved oxygen (mg/L) | 8.0 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 4660 | | |
| | Temperature (°C) | 25.3 | 25.6 | 25.5 |
| 3000 mg/L | pH (S.U.) | 7.82 | 7.89 | |
| | Dissolved oxygen (mg/L) | 8.0 | 8.2 | |
| | Conductivity (µmhos/cm) | 5550 | | |
| | Temperature (°C) | 25.6 | 25.5 | |

AP 03-20-25

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measure 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 o test specific bench sheets and transcribed to this bench sheet.

### *Chemical analyses:*

| Parameter | Reporting limit | Method number | Meter | Serial numb |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O G-2021 | YSI Model 52CE | 08A100271 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2021 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2021 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2021 | Not applicable | Not applicabl |
| Temperature | 0.1 °C | SM 2550B-2010 | Digital Thermometer | 130664 |

# 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 # 11

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 03-18-25 | *0917 | JP | 1135 | JP | 2B | Black | 03-13-25 |
| 24 | 03-19-25 | | | 1133 | JP | | | |
| 48 Termination | 03-20-25 | | | 1135 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 03-11-25 A |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 03-17-25 1313 to 03-18-25 0917 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.86 |
| | Temperature (°C): 24.1°C |

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

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

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 1 4d 2003-19-25 | 2 3d | 0 5d | 2 3d | 0 5d | 0 5d | 0 5d | 0 5d |
| 48 Termination | 5 | 2 3d | 4 1d | 4 1d | 0 4d | 0 3d | 0 | 1 1d | 0 | 0 | 0 | 0 |
| Mean Survival | 75% | | | | 5% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Probit |
| Lower 95% confidence limit (mg NaCl/L) | 2021.4 |
| Upper 95% confidence limit (mg NaCl/L) | 2252.2 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 2134.0 |

Comments:

Test Reviewed by: [signature]

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 3/18/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 3/20/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 0.2000 | 0.4000 | 0.0000 | 0.4000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

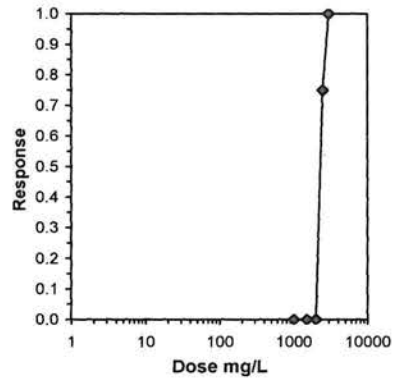
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2500 | 0.2500 | 0.2500 | 0.5146 | 0.2255 | 0.6847 | 42.578 | 4 | 10.00 | 10.00 | 15 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.5773 | 0.868 | -1.2815 | 7.35578 |
| Equality of variance cannot be confirmed | | | | |

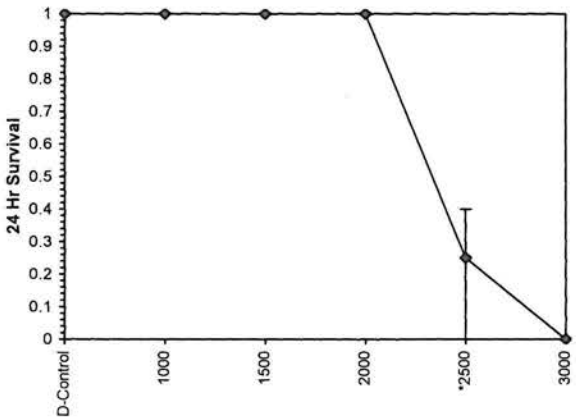
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2000 | 2500 | 2236.07 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2352.32 | 2261.76 | 2446.51 |
| 5.0% | 2343.17 | 2244.68 | 2445.98 |
| 10.0% | 2334.93 | 2229.9 | 2444.9 |
| 20.0% | 2322.88 | 2211.45 | 2439.93 |
| Auto-0.0% | 2352.32 | 2261.76 | 2446.51 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 3/18/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 3/20/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 0.4000 | 0.8000 | 0.8000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.2000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| Conc-mg/L | Mean | N-Mean | Transform: Arcsin Square Root Mean | Min | Max | CV% | N | Rank Sum | 1-Tailed Critical | Number Resp | Total Number |
|---|---|---|---|---|---|---|---|---|---|---|---|
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 0.7500 | 0.7500 | 1.0611 | 0.6847 | 1.3453 | 25.905 | 4 | 12.00 | 10.00 | 5 | 20 |
| *2500 | 0.0500 | 0.0500 | 0.2850 | 0.2255 | 0.4636 | 41.771 | 4 | 10.00 | 10.00 | 19 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

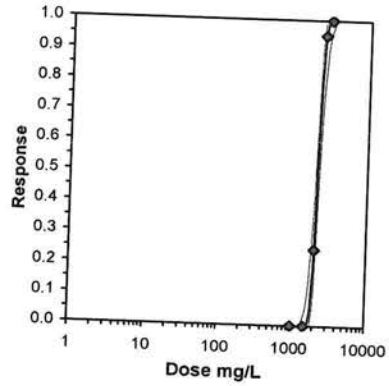
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.71358 | 0.868 | -0.8701 | 6.41185 |
| Equality of variance cannot be confirmed | | | | |

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

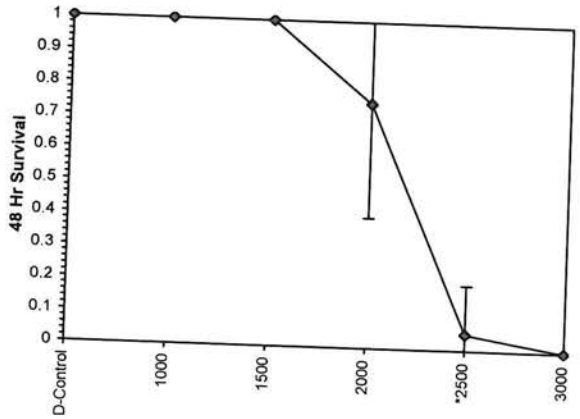
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 24.0509 | 5.63628 | 13.0038 | 35.098 | 0 | 0.00647 | 7.81472 | 0.99986 | 3.3292 | 0.04158 | 3 |
| Intercept | -75.07 | 18.7675 | -111.85 | -38.286 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1707.94 | 1397.63 | 1852.19 |
| EC05 | 3.355 | 1823.09 | 1570.73 | 1944.47 |
| EC10 | 3.718 | 1887.62 | 1669.45 | 1998.11 |
| EC15 | 3.964 | 1932.44 | 1737.98 | 2036.96 |
| EC20 | 4.158 | 1968.82 | 1793.07 | 2069.96 |
| EC25 | 4.326 | 2000.58 | 1840.37 | 2100.23 |
| EC40 | 4.747 | 2082.89 | 1956.48 | 2188.17 |
| EC50 | 5.000 | 2134.02 | 2021.4 | 2252.19 |
| EC60 | 5.253 | 2186.42 | 2080.92 | 2326.51 |
| EC75 | 5.674 | 2276.37 | 2168.53 | 2472.74 |
| EC80 | 5.842 | 2313.09 | 2200.38 | 2537.82 |
| EC85 | 6.036 | 2356.64 | 2236.14 | 2618.12 |
| EC90 | 6.282 | 2412.59 | 2279.73 | 2725.46 |
| EC95 | 6.645 | 2497.98 | 2342.72 | 2896.63 |
| EC99 | 7.326 | 2666.4 | 2459.54 | 3255.23 |




### Dose-Response Plot



# 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 #** 12

***Dilution Preparation:***

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2368

***Chemical Analyses:***

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | JP | JP | JP |
| Control, MHSW | pH (S.U.) | 7.82 | 7.87 | 7.95 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 310 | | |
| | Alkalinity (mg/L $CaCO_3$) | 60 | | |
| | Hardness (mg/L $CaCO_3$) | 87 | | |
| | Temperature (°C) | 25.7 | 25.3 | 25.6 |
| 1000 mg/L | pH (S.U.) | 7.92 | 7.91 | 7.98 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.2 |
| | Conductivity (µmhos/cm) | 2140 | | |
| | Temperature (°C) | 25.7 | 25.5 | 25.7 |
| 1500 mg/L | pH (S.U.) | 7.92 | 7.93 | 7.98 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.2 |
| | Conductivity (µmhos/cm) | 3010 | | |
| | Temperature (°C) | 25.6 | 25.3 | 25.6 |
| 2000 mg/L | pH (S.U.) | 7.91 | 7.91 | 7.97 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.2 |
| | Conductivity (µmhos/cm) | 3840 | | |
| | Temperature (°C) | 25.5 | 25.4 | 25.6 |
| 2500 mg/L | pH (S.U.) | 7.91 | 7.92 | 7.99 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | 8.2 |
| | Conductivity (µmhos/cm) | 4690 | | |
| | Temperature (°C) | 25.5 | 25.3 | 25.5 |
| 3000 mg/L | pH (S.U.) | 7.91 | 7.91 | |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | |
| | Conductivity (µmhos/cm) | 5520 | | |
| | Temperature (°C) | 25.3 | 25.3 | |

JP 03-26-25

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 |

# 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 # 12

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 03-25-25 | *0922 | JP | 1152 | JP | 2B | Green | 03-18-25 |
| 24 | 03-26-25 | | | 1200 | JP | | | |
| 48 Termination | 03-27-25 | | | 1153 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 03-18-25 A+B |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 03-24-25 1320 to 03-25-25 0922 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.76 |
| | Temperature (°C): 24.1 C |

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

| Hours | Control | | | | 1000 mg/L | | | | 1500 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 48 Termination | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 d | 5 | 4 d | 5 |
| Mean Survival | 100% | | | | 100% | | | | 95% | | | |

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

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Probit |
| Lower 95% confidence limit (mg NaCl/L) | 1741.5 |
| Upper 95% confidence limit (mg NaCl/L) | 2066.8 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1932.8 |

Comments:

Test Reviewed by: _____

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 3/25/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 3/27/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 0.8000 | 0.0000 | 0.6000 | 1.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

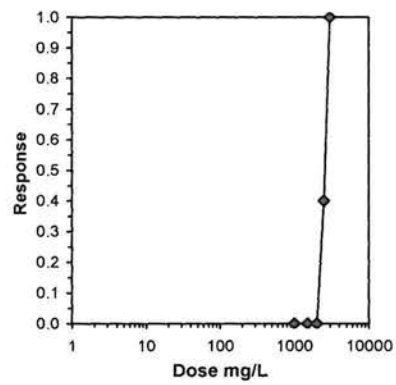
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2500 | 0.6000 | 0.6000 | 0.8910 | 0.2255 | 1.3453 | 54.058 | 4 | 12.00 | 10.00 | 8 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.51912 | 0.868 | -1.5922 | 9.43065 |
| Equality of variance cannot be confirmed | | | | |

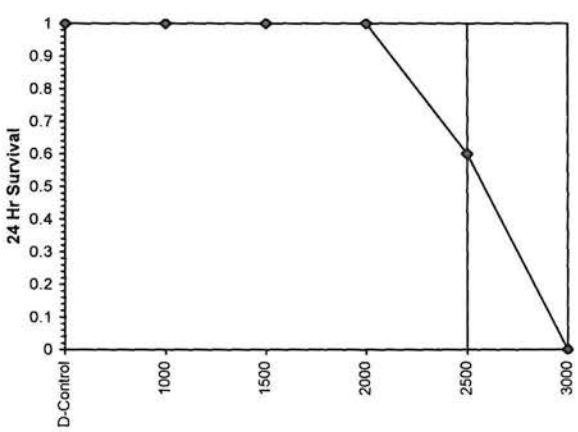
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2500 | 3000 | 2738.61 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2525.3 | 2415.59 | 2639.99 |
| 5.0% | 2532.97 | 2410.35 | 2661.83 |
| 10.0% | 2540.58 | 2400.44 | 2688.89 |
| 20.0% | 2555.41 | 2357.73 | 2769.66 |
| Auto-0.0% | 2525.3 | 2415.59 | 2639.99 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 3/25/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 3/27/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 0.8000 | 1.0000 | 0.8000 | 1.0000 |
| 2000 | 0.4000 | 0.6000 | 0.6000 | 0.6000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.9000 | 0.9000 | 1.2262 | 1.1071 | 1.3453 | 11.212 | 4 | 14.00 | 10.00 | 2 | 20 |
| *2000 | 0.5500 | 0.5500 | 0.8357 | 0.6847 | 0.8861 | 12.047 | 4 | 10.00 | 10.00 | 9 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

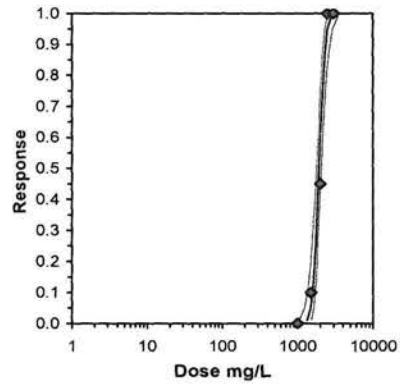
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.8659 | 0.844 | -0.527 | 0.25925 |
| Equality of variance cannot be confirmed | | | | |

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

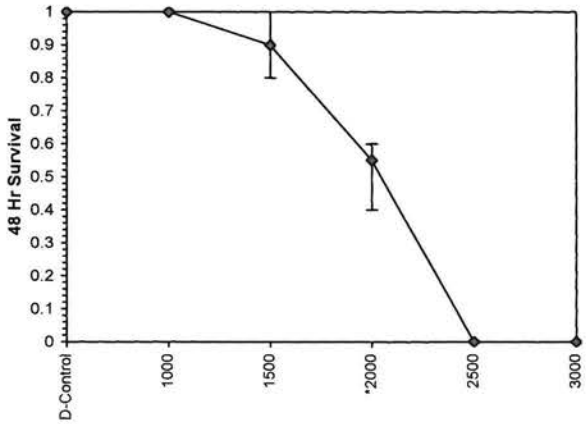
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 14.5838 | 2.74442 | 9.20477 | 19.9629 | 0 | 3.47816 | 7.81472 | 0.32361 | 3.28618 | 0.06857 | 6 |
| Intercept | -42.925 | 9.05472 | -60.672 | -25.178 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1338.65 | 1047.53 | 1510.35 |
| EC05 | 3.355 | 1490.72 | 1236.47 | 1641.49 |
| EC10 | 3.718 | 1578.73 | 1348.89 | 1718.35 |
| EC15 | 3.964 | 1641.03 | 1429.15 | 1773.86 |
| EC20 | 4.158 | 1692.29 | 1495.2 | 1820.62 |
| EC25 | 4.326 | 1737.54 | 1553.2 | 1863.04 |
| EC40 | 4.747 | 1857 | 1702.3 | 1982.72 |
| EC50 | 5.000 | 1932.79 | 1791.47 | 2066.82 |
| EC60 | 5.253 | 2011.66 | 1877.94 | 2162.95 |
| EC75 | 5.674 | 2149.97 | 2013.33 | 2353.21 |
| EC80 | 5.842 | 2207.46 | 2064.48 | 2439.46 |
| EC85 | 6.036 | 2276.41 | 2122.87 | 2547.44 |
| EC90 | 6.282 | 2366.24 | 2195.23 | 2694.37 |
| EC95 | 6.645 | 2505.93 | 2301.89 | 2934.4 |
| EC99 | 7.326 | 2790.61 | 2505.89 | 3457.93 |




### Dose-Response Plot



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 # 13 HT 20 24-15-25

### *Dilution Preparation:*

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| 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 stock solution was used to prepare the concentrations evaluated for toxicity.

Stock solution INSS #: 2368

### *Chemical Analyses:*

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | JP JK | JP | JP |
| Control, MHSW | pH (S.U.) | 7.86 | 7.95 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 306 | | |
| | Alkalinity (mg/L $CaCO_3$) | ~~81~~ 78 | | |
| | Hardness (mg/L $CaCO_3$) | 81 | | |
| | Temperature (°C) | 25.7 | 25.8 | 24.7 |
| 1000 mg/L | pH (S.U.) | 7.93 | 7.92 | 7.93 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 2060 | | |
| | Temperature (°C) | 25.7 | 25.8 | 24.6 |
| 1500 mg/L | pH (S.U.) | 7.93 | 7.95 | 7.94 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 2910 | | |
| | Temperature (°C) | 25.5 | 25.6 | 24.5 |
| 2000 mg/L | pH (S.U.) | 7.93 | 7.95 | 7.93 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 3790 | | |
| | Temperature (°C) | 25.5 | 25.7 | 24.6 |
| 2500 mg/L | pH (S.U.) | 7.93 | 7.92 | 7.93 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 4590 | | |
| | Temperature (°C) | 25.6 | 25.7 | 24.7 |
| 3000 mg/L | pH (S.U.) | 7.92 | 7.91 | 7.93 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | 8.2 |
| | Conductivity (µmhos/cm) | 5450 | | |
| | Temperature (°C) | 25.3 | 25.7 | 24.6 |

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measurer 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 o test specific bench sheets and transcribed to this bench sheet.

### *Chemical analyses:*

| Parameter | Reporting limit | Method number | Meter | Serial numb |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O G-2021 | YSI Model 52CE | 08A100271 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2021 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2021 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2021 | Not applicable | Not applicabl |
| Temperature | 0.1 °C | SM 2550B-2010 | Digital Thermometer | 130664 |

# 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 # 13 ~~14~~ 2024-15-25

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 04-15-25 | * 0923 | JP | 1135 | JP | 2B | Green | 04-12-25A |
| 24 | 04-16-25 | | | 1127 | JP | | | |
| 48 Termination | 04-17-25 | | | 1130 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 04-08-25 A |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 04-14-25 1145 to 04-15-25 0923 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl Information: | pH (S.U.): 7.70 |
| | Temperature (°C): 24.6°C |

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

| Hours | Control | | | | 1000 mg/L | | | | 1500 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 48 Termination | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3 2d |
| Mean Survival | 100 % | | | | 100 % | | | | 90 % | | | |

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 2 3d | 2 3d | 1 4d | 0 5d | 0 5d | 0 5d | 1 4d | 0 5d |
| 48 Termination | 2 3d | 1 4d | 5 | 3 2d | 0 2d | 0 2d | 0 1d | 0 | 0 | 0 | 0 u | 0 |
| Mean Survival | 55 % | | | | 0 % | | | | 0 % | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Probit |
| Lower 95% confidence limit (mg NaCl/L) | 1791.5 |
| Upper 95% confidence limit (mg NaCl/L) | 2066.8 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1932.8 |

Comments:

Test Reviewed by: JP

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 4/15/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 4/17/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 0.4000 | 0.4000 | 0.2000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.2000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Rank Sum | 1-Tailed Critical | Number Resp | Total Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2500 | 0.2500 | 0.2500 | 0.5146 | 0.2255 | 0.6847 | 42.578 | 4 | 10.00 | 10.00 | 15 | 20 |
| *3000 | 0.0500 | 0.0500 | 0.2850 | 0.2255 | 0.4636 | 41.771 | 4 | 10.00 | 10.00 | 19 | 20 |

**Auxiliary Tests**

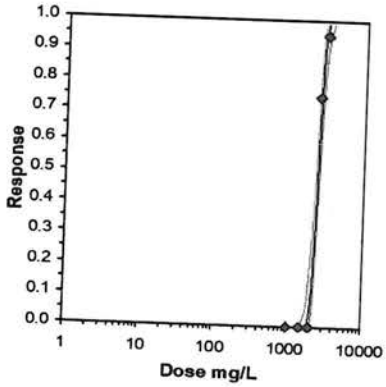
| | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.70201 | 0.884 | -0.6104 | 4.90861 |
| Equality of variance cannot be confirmed | | | | |

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

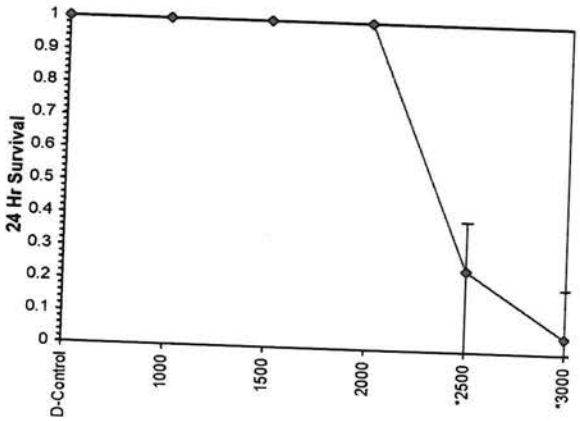
**Maximum Likelihood-Probit**

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 22.9104 | 4.90267 | 13.3012 | 32.5197 | 0 | 3.42843 | 7.81472 | 0.33016 | 3.38149 | 0.04365 | 6 |
| Intercept | -72.471 | 16.6099 | -105.03 | -39.916 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1905.22 | 1576.49 | 2071.29 |
| EC05 | 3.355 | 2040.28 | 1767.72 | 2181.29 |
| EC10 | 3.718 | 2116.16 | 1876.97 | 2244.67 |
| EC15 | 3.964 | 2168.94 | 1953.08 | 2290.11 |
| EC20 | 4.158 | 2211.82 | 2014.57 | 2328.26 |
| EC25 | 4.326 | 2249.29 | 2067.69 | 2362.83 |
| EC40 | 4.747 | 2346.53 | 2200.42 | 2460.51 |
| EC50 | 5.000 | 2407.05 | 2276.87 | 2529.48 |
| EC60 | 5.253 | 2469.13 | 2348.7 | 2608.43 |
| EC75 | 5.674 | 2575.88 | 2456.66 | 2763.59 |
| EC80 | 5.842 | 2619.51 | 2496.17 | 2833.01 |
| EC85 | 6.036 | 2671.31 | 2540.54 | 2919 |
| EC90 | 6.282 | 2737.93 | 2594.56 | 3034.32 |
| EC95 | 6.645 | 2839.75 | 2672.55 | 3218.73 |
| EC99 | 7.326 | 3041.07 | 2817.14 | 3605.74 |




**Dose-Response Plot**



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 4/15/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 4/17/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 0.6000 |
| 2000 | 0.4000 | 0.2000 | 1.0000 | 0.6000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.9000 | 0.9000 | 1.2305 | 0.8861 | 1.3453 | 18.660 | 4 | 16.00 | 10.00 | 2 | 20 |
| 2000 | 0.5500 | 0.5500 | 0.8449 | 0.4636 | 1.3453 | 44.446 | 4 | 12.00 | 10.00 | 9 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

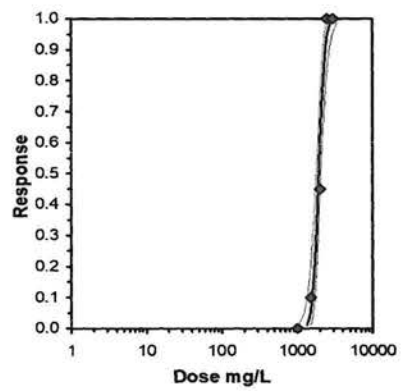
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.82954 | 0.844 | 0.29445 | 2.86452 |
| Equality of variance cannot be confirmed | | | | |

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

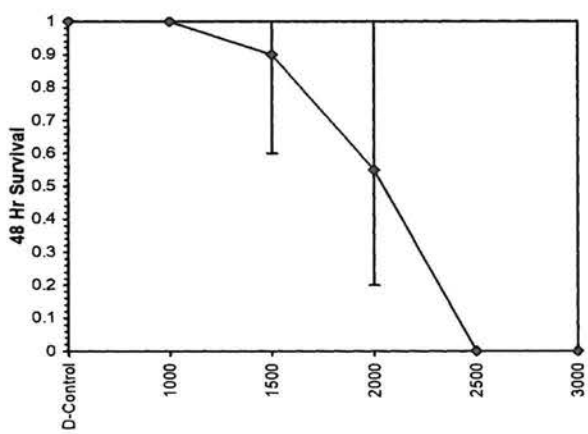
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 14.5838 | 2.74442 | 9.20477 | 19.9629 | 0 | 3.47816 | 7.81472 | 0.32361 | 3.28618 | 0.06857 | 6 |
| Intercept | -42.925 | 9.05472 | -60.672 | -25.178 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1338.65 | 1047.53 | 1510.35 |
| EC05 | 3.355 | 1490.72 | 1236.47 | 1641.49 |
| EC10 | 3.718 | 1578.73 | 1348.89 | 1718.35 |
| EC15 | 3.964 | 1641.03 | 1429.15 | 1773.86 |
| EC20 | 4.158 | 1692.29 | 1495.2 | 1820.62 |
| EC25 | 4.326 | 1737.54 | 1553.2 | 1863.04 |
| EC40 | 4.747 | 1857 | 1702.3 | 1982.72 |
| EC50 | 5.000 | 1932.79 | 1791.47 | 2066.82 |
| EC60 | 5.253 | 2011.66 | 1877.94 | 2162.95 |
| EC75 | 5.674 | 2149.97 | 2013.33 | 2353.21 |
| EC80 | 5.842 | 2207.46 | 2064.48 | 2439.46 |
| EC85 | 6.036 | 2276.41 | 2122.87 | 2547.44 |
| EC90 | 6.282 | 2366.24 | 2195.23 | 2694.37 |
| EC95 | 6.645 | 2505.93 | 2301.89 | 2934.4 |
| EC99 | 7.326 | 2790.61 | 2505.89 | 3457.93 |




### Dose-Response Plot



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 # 14

### *Dilution Preparation:*

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| 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/ stock solution was used to prepare the concentrations evaluated for toxicity.

**Stock solution INSS #:** 2368

### *Chemical Analyses:*

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | [illegible] | [illegible] | [illegible] |
| Control, MHSW | pH (S.U.) | 7.80 | 7.52 | 7.88 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.1 | 8.3 |
| | Conductivity (µmhos/cm) | 300 | | |
| | Alkalinity (mg/L $CaCO_3$) | 58 | | |
| | Hardness (mg/L $CaCO_3$) | 81 | | |
| | Temperature (°C) | 25.6 | 25.7 | 25.9 |
| 1000 mg/L | pH (S.U.) | 7.88 | 7.53 | 7.92 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.4 |
| | Conductivity (µmhos/cm) | 2020 | | |
| | Temperature (°C) | 25.6 | 25.6 | 25.9 |
| 1500 mg/L | pH (S.U.) | 7.89 | 7.56 | 7.91 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.4 |
| | Conductivity (µmhos/cm) | 2880 | | |
| | Temperature (°C) | 25.4 | 25.3 | 25.8 |
| 2000 mg/L | pH (S.U.) | 7.90 | 7.58 | 7.91 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.4 |
| | Conductivity (µmhos/cm) | 3740 | | |
| | Temperature (°C) | 25.5 | 25.4 | 25.8 |
| 2500 mg/L | pH (S.U.) | 7.89 | 7.61 | 7.91 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.4 |
| | Conductivity (µmhos/cm) | 4520 | | |
| | Temperature (°C) | 25.4 | 25.4 | 25.7 |
| 3000 mg/L | pH (S.U.) | 7.89 | 7.61 | 7.91 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.4 |
| | Conductivity (µmhos/cm) | 5450 | | |
| | Temperature (°C) | 25.3 | 25.5 | 25.6 |

Analyst identified for each day, performed pH, dissolved oxygen and conductivity measuren 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 or test specific bench sheets and transcribed to this bench sheet.

### *Chemical analyses:*

| Parameter | Reporting limit | Method number | Meter | Serial numb |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O G-2021 | YSI Model 52CE | 08A100271 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2021 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2021 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2021 | Not applicable | Not applicabl |
| Temperature | 0.1 °C | SM 2550B-2010 | Digital Thermometer | 13066 |

ETC

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

***Ceriodaphnia dubia*** **Sodium Chloride Acute Reference Toxicant Test**

CdNaClAC # 14

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 04-22-25 | * 0925 | zp | 1140 | zp | 1D | Green | ~~04-22-25~~ A ~~04-22-25~~ 04-21-25 |
| 24 | 04-23-25 | | | 1130 | zp | | | |
| 48 Termination | 04-24-25 | | | 1209 | zp | | | |

*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): | 04-15-25 A |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 04-21-25 1205 to 04-22-25 0925 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.61 |
| | Temperature (°C): 24.1°C |

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

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

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 4 d | 3 d | 3 d | 3 d | 1 d | 0 d | 0 d | 1 d | 0 d |
| 48 Termination | 1 d | 3 d | 1 d | 0 | 0 d | 0 d | 0 d | 0 d | 0 | 0 | 0 d | 0 |
| Mean Survival | 40% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Spearman-Karber |
| Lower 95% confidence limit (mg NaCl/L) | 1814.0 |
| Upper 95% confidence limit (mg NaCl/L) | 2028.8 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1918.4 |

Comments:

Test Reviewed by:

Environmental Testing Solutions, Inc.

## Acute Daphnid Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 4/22/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 4/24/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 1.0000 | 1.0000 | 0.8000 |
| 2500 | 0.6000 | 0.6000 | 0.6000 | 0.2000 |
| 3000 | 0.0000 | 0.0000 | 0.2000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 0.9500 | 0.9500 | 1.2857 | 1.1071 | 1.3453 | 9.261 | 4 | 16.00 | 10.00 | 1 | 20 |
| *2500 | 0.5000 | 0.5000 | 0.7805 | 0.4636 | 0.8861 | 27.063 | 4 | 10.00 | 10.00 | 10 | 20 |
| *3000 | 0.0500 | 0.0500 | 0.2850 | 0.2255 | 0.4636 | 41.771 | 4 | 10.00 | 10.00 | 19 | 20 |

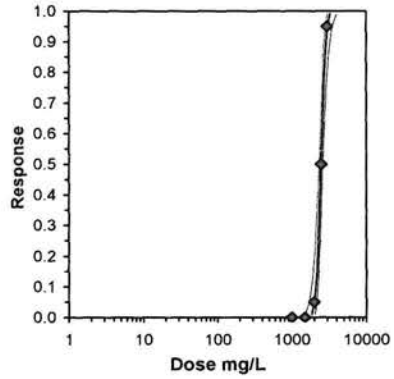
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.82971 | 0.884 | -1.4441 | 4.39401 |
| Equality of variance cannot be confirmed | | | | |

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

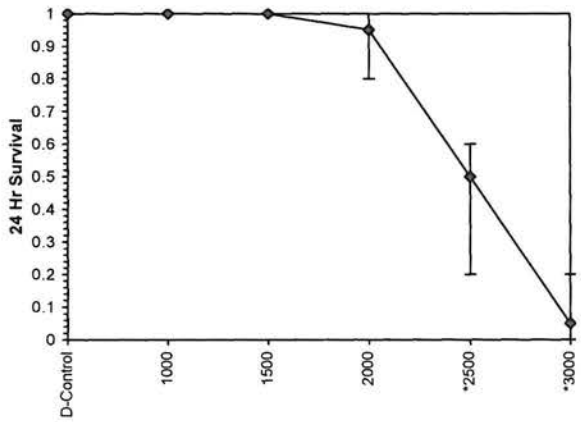
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 18.6319 | 3.79507 | 11.1936 | 26.0703 | 0 | 0.1442 | 7.81472 | 0.98605 | 3.39427 | 0.05367 | 3 |
| Intercept | -58.242 | 12.9007 | -83.527 | -32.957 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1859.58 | 1508.9 | 2046.84 |
| EC05 | 3.355 | 2022.98 | 1729.36 | 2182.13 |
| EC10 | 3.718 | 2115.87 | 1857.5 | 2260.62 |
| EC15 | 3.964 | 2180.95 | 1947.66 | 2317.11 |
| EC20 | 4.158 | 2234.09 | 2020.98 | 2364.69 |
| EC25 | 4.326 | 2280.72 | 2084.65 | 2407.93 |
| EC40 | 4.747 | 2402.56 | 2244.72 | 2531.01 |
| EC50 | 5.000 | 2478.98 | 2337.29 | 2618.74 |
| EC60 | 5.253 | 2557.82 | 2424.46 | 2719.83 |
| EC75 | 5.674 | 2694.47 | 2556.54 | 2919.32 |
| EC80 | 5.842 | 2750.7 | 2605.5 | 3008.74 |
| EC85 | 6.036 | 2817.73 | 2661 | 3119.66 |
| EC90 | 6.282 | 2904.39 | 2729.32 | 3268.87 |
| EC95 | 6.645 | 3037.76 | 2829.31 | 3508.84 |
| EC99 | 7.326 | 3304.69 | 3018.18 | 4019.05 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

## Acute Daphnid Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 4/22/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 4/24/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.2000 | 0.6000 | 0.2000 | 0.6000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

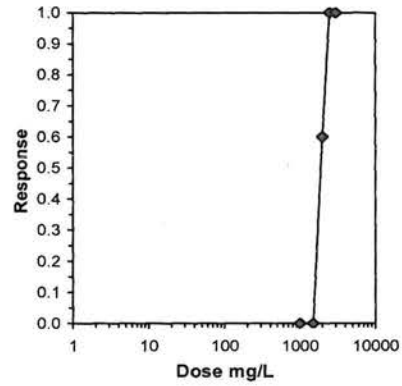
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2000 | 0.4000 | 0.4000 | 0.6749 | 0.4636 | 0.8861 | 36.139 | 4 | 10.00 | 10.00 | 12 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.69656 | 0.844 | -8E-16 | 1.8956 |
| Equality of variance cannot be confirmed | | | | |

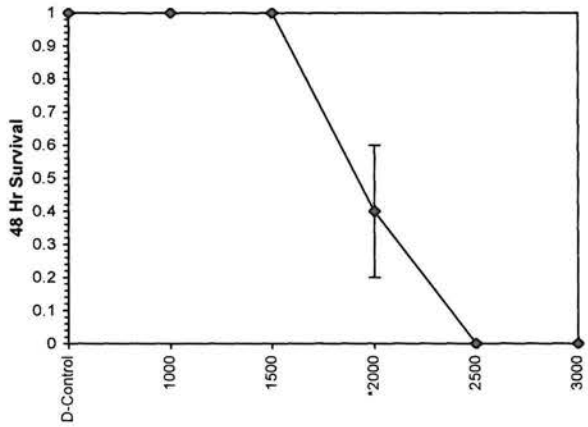
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 1500 | 2000 | 1732.05 | |
| Treatments vs D-Control | | | | |

### Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 1918.36 | 1813.96 | 2028.77 |
| 5.0% | 1916.57 | 1801.36 | 2039.14 |
| 10.0% | 1914.79 | 1786.89 | 2051.85 |
| 20.0% | 1911.36 | 1748.75 | 2089.09 |
| Auto-0.0% | 1918.36 | 1813.96 | 2028.77 |




### Dose-Response Plot



# 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 #** 15

***Dilution Preparation:***

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2375

***Chemical Analyses:***

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | JP Ens | JP | JP |
| Control, MHSW | pH (S.U.) | 7.83 | 7.87 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.2 | 8.3 |
| | Conductivity (µmhos/cm) | 301 | | |
| | Alkalinity (mg/L $CaCO_3$) | 60 | | |
| | Hardness (mg/L $CaCO_3$) | 86 | | |
| | Temperature (°C) | 25.6 | 25.7 | 25.6 |
| 1000 mg/L | pH (S.U.) | 7.91 | 7.92 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.2 | 8.3 |
| | Conductivity (µmhos/cm) | 1980 | | |
| | Temperature (°C) | 25.6 | 25.6 | 25.5 |
| 1500 mg/L | pH (S.U.) | 7.91 | 7.94 | 7.93 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 2850 | | |
| | Temperature (°C) | 25.4 | 25.7 | 25.7 |
| 2000 mg/L | pH (S.U.) | 7.91 | 7.94 | 7.92 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 3700 | | |
| | Temperature (°C) | 25.2 | 25.8 | 25.7 |
| 2500 mg/L | pH (S.U.) | 7.91 | 7.94 | 7.95 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.3 | 8.1 |
| | Conductivity (µmhos/cm) | 4580 | | |
| | Temperature (°C) | 25.1 | 24.7 | 25.6 |
| 3000 mg/L | pH (S.U.) | 7.91 | 7.95 | |
| | Dissolved oxygen (mg/L) | 8.3 | 8.3 | |
| | Conductivity (µmhos/cm) | 5470 | | |
| | Temperature (°C) | 25.3 | 25.3 | |

JP 05-01-25

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

***Chemical analyses:***

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O 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 | 1306645 |

# 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 # 15

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 04-24-25 | *0918 | JP | 1135 | JP | 2B | Rec | 04-23-25 A |
| 24 | 04-30-25 | | | 1138 | JP | | | |
| 48 Termination | 05-01-25 | | | 1137 | JP | | | |

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

***Test Organism Information:***

| | |
|---|---|
| Organism Source: | In-house Culture |
| Source (organisms were pooled): | 04-22-25 A |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 04-28-25 1209 to 04-29-25 0918 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.64 |
| | Temperature (°C): 25.1°C |

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

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

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 4 1d | 5 | 5 | 4 1d | 2 3d 2d 5 | 5 | 2 3d | 0 5d | 0 5d | 0 5d | 0 5d |
| 48 Termination | 2 3d | 1 3d | 4 1d | 1 4d | 0 | 0 3d | 0 5d | 0 2d | 0 | 0 | 0 | 0 |
| Mean Survival | 40% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Spearman-Karber |
| Lower 95% confidence limit (mg NaCl/L) | 1814.0 |
| Upper 95% confidence limit (mg NaCl/L) | 2028.9 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1918.4 |

Comments:

Test Reviewed by:

# Statistical Analyses

**Acute Daphnid Test-24 Hr Survival**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 4/29/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/1/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 0.8000 | 1.0000 | 1.0000 |
| 2500 | 0.8000 | 0.6000 | 1.0000 | 0.4000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 0.9500 | 0.9500 | 1.2857 | 1.1071 | 1.3453 | 9.261 | 4 | 16.00 | 10.00 | 1 | 20 |
| 2500 | 0.7000 | 0.7000 | 1.0058 | 0.6847 | 1.3453 | 28.293 | 4 | 12.00 | 10.00 | 6 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

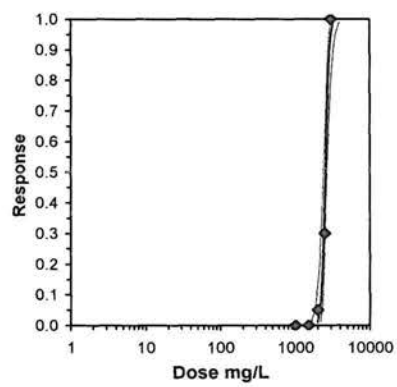
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.77719 | 0.868 | 0.00888 | 4.54899 |
| Equality of variance cannot be confirmed | | | | |

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

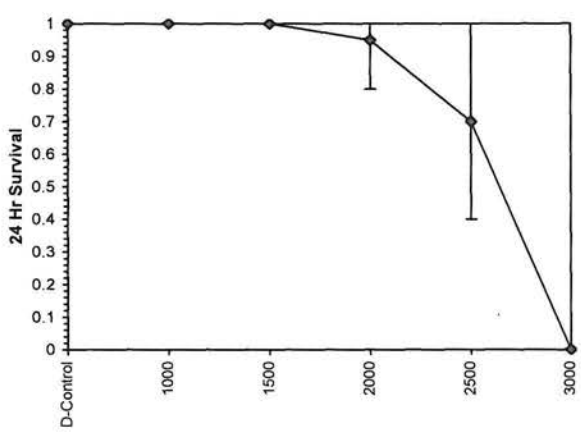
**Maximum Likelihood-Probit**

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 21.7684 | 4.79926 | 12.3619 | 31.175 | 0 | 5.29448 | 7.81472 | 0.15146 | 3.40491 | 0.04594 | 6 |
| Intercept | -69.12 | 16.3672 | -101.2 | -37.04 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1986.28 | 1616.64 | 2167.93 |
| EC05 | 3.355 | 2134.74 | 1829.26 | 2287.56 |
| EC10 | 3.718 | 2218.38 | 1951.7 | 2356.55 |
| EC15 | 3.964 | 2276.65 | 2037.39 | 2406.06 |
| EC20 | 4.158 | 2324.05 | 2106.82 | 2447.72 |
| EC25 | 4.326 | 2365.5 | 2166.93 | 2485.57 |
| EC40 | 4.747 | 2473.26 | 2317.14 | 2593.55 |
| EC50 | 5.000 | 2540.43 | 2403.13 | 2671.1 |
| EC60 | 5.253 | 2609.43 | 2483.06 | 2761.22 |
| EC75 | 5.674 | 2728.3 | 2601.43 | 2940.71 |
| EC80 | 5.842 | 2776.96 | 2644.45 | 3021.42 |
| EC85 | 6.036 | 2834.78 | 2692.71 | 3121.5 |
| EC90 | 6.282 | 2909.24 | 2751.55 | 3255.87 |
| EC95 | 6.645 | 3023.21 | 2836.72 | 3471.13 |
| EC99 | 7.326 | 3249.19 | 2995.46 | 3924.76 |




**Dose-Response Plot**



# Statistical Analyses

**Acute Daphnid Test-48 Hr Survival**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 4/29/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/1/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.4000 | 0.2000 | 0.8000 | 0.2000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

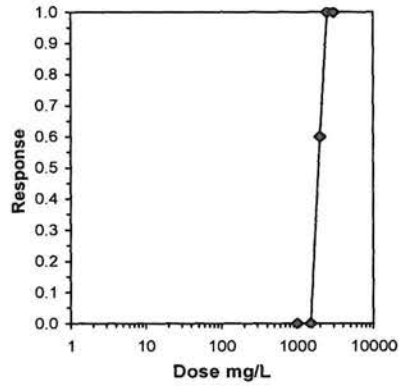
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2000 | 0.4000 | 0.4000 | 0.6798 | 0.4636 | 1.1071 | 44.627 | 4 | 10.00 | 10.00 | 12 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.57398 | 0.844 | 1.76519 | 7.38425 |
| Equality of variance cannot be confirmed | | | | |

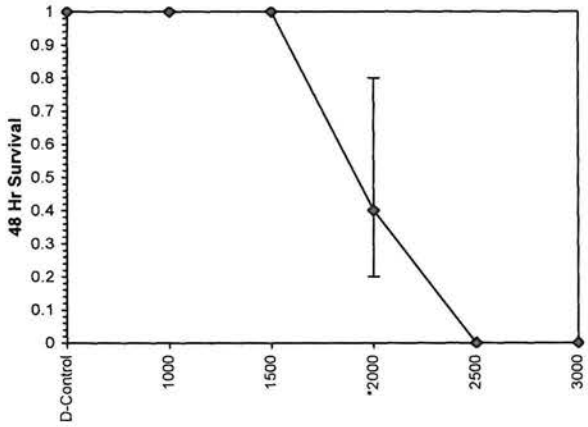
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 1500 | 2000 | 1732.05 | |
| Treatments vs D-Control | | | | |

**Trimmed Spearman-Karber**

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 1918.36 | 1813.96 | 2028.77 |
| 5.0% | 1916.57 | 1801.36 | 2039.14 |
| 10.0% | 1914.79 | 1786.89 | 2051.85 |
| 20.0% | 1911.36 | 1748.75 | 2089.09 |
| Auto-0.0% | 1918.36 | 1813.96 | 2028.77 |




**Dose-Response Plot**



# 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 #** 16

### *Dilution Preparation:*

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198.0 | 197.0 | 196.0 | 195.0 | 194.0 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2375

### *Chemical Analyses:*

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | VP | VP Em | VP Em |
| Control, MHSW | pH (S.U.) | 8.13 | 7.83 | 7.88 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 8.1 |
| | Conductivity (µmhos/cm) | 296 | | |
| | Alkalinity (mg/L $CaCO_3$) | 58 | | |
| | Hardness (mg/L $CaCO_3$) | 88 | | |
| | Temperature (°C) | 24.6 | 24.9 | 25.2 |
| 1000 mg/L | pH (S.U.) | 8.14 | 7.85 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 2170 | | |
| | Temperature (°C) | 25.1 | 25.0 | 25.2 |
| 1500 mg/L | pH (S.U.) | 8.11 | 7.85 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 3060 | | |
| | Temperature (°C) | 25.0 | 25.1 | 25.3 |
| 2000 mg/L | pH (S.U.) | 8.10 | 7.86 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 3920 | | |
| | Temperature (°C) | 24.4 | 25.0 | 25.2 |
| 2500 mg/L | pH (S.U.) | 8.09 | 7.86 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 4830 | | |
| | Temperature (°C) | 24.7 | 25.0 | 25.3 |
| 3000 mg/L | pH (S.U.) | 8.09 | 7.86 | |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | |
| | Conductivity (µmhos/cm) | 6070 | | |
| | Temperature (°C) | 24.1 | 24.8 | |

2005-07-25

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 | 1306647 |

# 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 # 17 16 ap 05-06-25

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 05-06-25 | *0906 | ap | 1132 | ap | 2C | Rec | 04-30-25A |
| 24 | 05-07-25 | | | 1137 | ap | | | |
| 48 Termination | 05-08-25 | | | 1126 | ap | | | |

*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): | 04-29-25 A |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 05-05-25 1330 to 05-06-25 0906 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 8.03 |
| | Temperature (°C): 25.7°C |

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

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

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 1 4d | 0 5d | 3 2d | 0 5d | 0 5d | 0 5d | 0 5d | 0 5d |
| 48 Termination | 2 3d | 0 5d | 2 3d | 2 3d | 0 1d | 0 | 0 3d | 0 | 0 | 0 | 0 | 0 |
| Mean Survival | 30% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | Spearman-Karber |
| Lower 95% confidence limit (mg NaCl/L) | 1774.6 |
| Upper 95% confidence limit (mg NaCl/L) | 1989.8 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1870.0 |

Comments:

Test Reviewed by:

# Statistical Analyses

**Acute Daphnid Test-24 Hr Survival**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/6/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/8/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 0.2000 | 0.0000 | 0.6000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

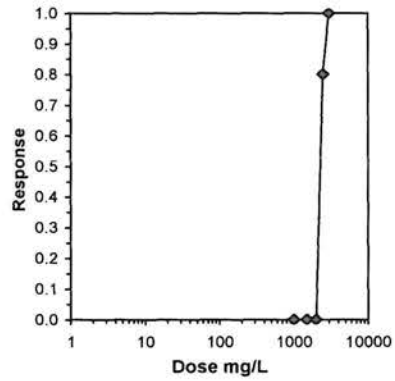
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2500 | 0.2000 | 0.2000 | 0.4502 | 0.2255 | 0.8861 | 69.198 | 4 | 10.00 | 10.00 | 16 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.52023 | 0.868 | 1.85413 | 9.13553 |
| Equality of variance cannot be confirmed | | | | |

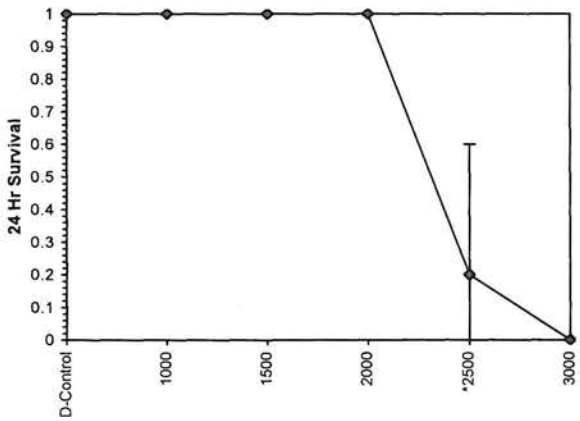
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2000 | 2500 | 2236.07 | |
| Treatments vs D-Control | | | | |

**Trimmed Spearman-Karber**

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2328.6 | 2245.66 | 2414.59 |
| 5.0% | 2317.57 | 2228.37 | 2410.35 |
| 10.0% | 2308.43 | 2216.52 | 2404.14 |
| 20.0% | 2299.32 | 2228.72 | 2372.15 |
| Auto-0.0% | 2328.6 | 2245.66 | 2414.59 |




**Dose-Response Plot**



# Statistical Analyses

Environmental Testing Solutions, Inc.

**Acute Daphnid Test-48 Hr Survival**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/6/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/8/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 0.4000 | 0.0000 | 0.4000 | 0.4000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

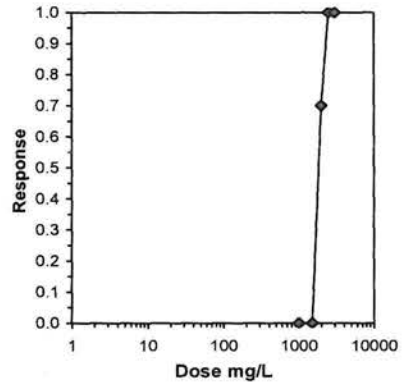
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2000 | 0.3000 | 0.3000 | 0.5699 | 0.2255 | 0.6847 | 40.287 | 4 | 10.00 | 10.00 | 14 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.56485 | 0.844 | -2.5555 | 9.36813 |
| Equality of variance cannot be confirmed | | | | |

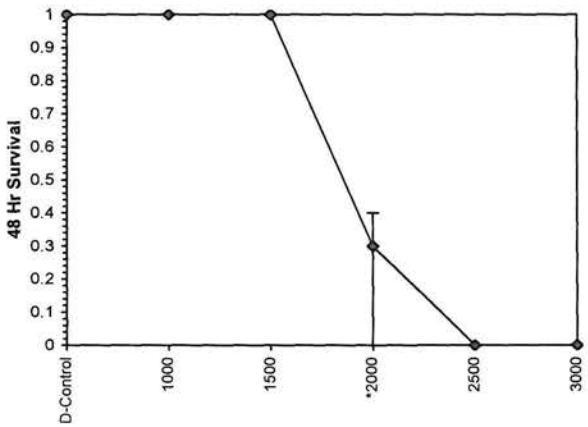
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 1500 | 2000 | 1732.05 | |
| Treatments vs D-Control | | | | |

**Trimmed Spearman-Karber**

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 1869.98 | 1774.62 | 1970.47 |
| 5.0% | 1863.6 | 1759.25 | 1974.14 |
| 10.0% | 1857.58 | 1744.15 | 1978.38 |
| 20.0% | 1847.3 | 1715 | 1989.81 |
| Auto-0.0% | 1869.98 | 1774.62 | 1970.47 |




**Dose-Response Plot**



# 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 # 17

### *Dilution Preparation:*

| Test concentrations (mg/L NaCl) | 1000 | 1500 | 2000 | 2500 | 3000 |
|---|---|---|---|---|---|
| mL Stock solution | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
| mL Dilution water (MHSW) | 198 | 197 | 196 | 195 | 194 |
| Total volume (mL) | 200 | 200 | 200 | 200 | 200 |

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

**Stock solution INSS #:** 2393

### *Chemical Analyses:*

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | XL | XL | XL |
| Control, MHSW | pH (S.U.) | 7.74 | 7.78 | 7.84 |
| | Dissolved oxygen (mg/L) | 8.6 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 294 | | |
| | Alkalinity (mg/L $CaCO_3$) | 62 | | |
| | Hardness (mg/L $CaCO_3$) | 88 | | |
| | Temperature (°C) | 24.9 | 24.8 | 25.2 |
| 1000 mg/L | pH (S.U.) | 7.88 | 7.80 | 7.83 |
| | Dissolved oxygen (mg/L) | 8.6 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 2060 | | |
| | Temperature (°C) | 25.2 | 25.0 | 25.2 |
| 1500 mg/L | pH (S.U.) | 7.87 | 7.81 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.6 | 8.2 | 8.3 |
| | Conductivity (µmhos/cm) | 2900 | | |
| | Temperature (°C) | 24.8 | 25.1 | 25.0 |
| 2000 mg/L | pH (S.U.) | 7.86 | 7.81 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.5 | 8.2 | 8.3 |
| | Conductivity (µmhos/cm) | 3720 | | |
| | Temperature (°C) | 24.8 | 24.7 | 24.8 |
| 2500 mg/L | pH (S.U.) | 7.85 | 7.81 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.5 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 4510 | | |
| | Temperature (°C) | 25.0 | 24.9 | 25.1 |
| 3000 mg/L | pH (S.U.) | 7.85 | 7.80 | |
| | Dissolved oxygen (mg/L) | 8.5 | 8.3 | |
| | Conductivity (µmhos/cm) | 5400 | | |
| | Temperature (°C) | 25.1 | 24.9 | |

06-04-25

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 |

# 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 # 17

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 06-03-25 | * 0530 | JK | 0730 | JK | 2B4 | GREEN | 05-28-25B |
| 24 | 06-04-25 | | | 0727 | JK | | | |
| 48 Termination | 06-05-25 | | | 0728 | 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 |
| Source (organisms were pooled): | 05-27-25 D |
| Age: | < 24-hours old |
| Date and time organisms were born between: | 06-02-25 0830 TU<br>06-03-25 0530 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 7.85 |
| | Temperature (°C): 25.0 |

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

| Hours | Control | | | | 1000 mg/L | | | | 1500 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 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 | 4 1d |
| Mean Survival | 100% | | | | 100% | | | | 95% | | | |

| Hours | 2000 mg/L | | | | 2500 mg/L | | | | 3000 mg/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | | | Replicate | | | | Replicate | | | |
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 Initiation | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 24 | 5 | 5 | 5 | 5 | 2 3d | 0 5d | 1 4d | 2 3d | 0 5d | 0 5d | 0 5d | 0 5d |
| 48 Termination | 3 2d | 1 4d | 1 4d | 2 3d | 0 2d | 0 | 0 1d | 0 2d | 0 | 0 | 0 | 0 |
| Mean Survival | 35% | | | | 0% | | | | 0% | | | |

Comment codes: d = dead, u = unhealthy

***Statistics:***

| | |
|---|---|
| Method | PROBIT |
| Lower 95% confidence limit (mg NaCl/L) | 1750.5 |
| Upper 95% confidence limit (mg NaCl/L) | 2001.1 |
| 48-hour $LC_{50}$ (mg NaCl/L) | 1879.9 |

Comments:

Test Reviewed by: A

# Statistical Analyses

**Acute Daphnid Test-24 Hr Survival**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 6/3/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 6/5/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2500 | 0.4000 | 0.0000 | 0.2000 | 0.4000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

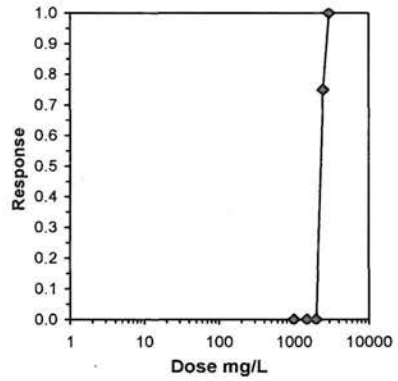
| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 2000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| *2500 | 0.2500 | 0.2500 | 0.5146 | 0.2255 | 0.6847 | 42.578 | 4 | 10.00 | 10.00 | 15 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.5773 | 0.868 | -1.2815 | 7.35578 |
| Equality of variance cannot be confirmed | | | | |

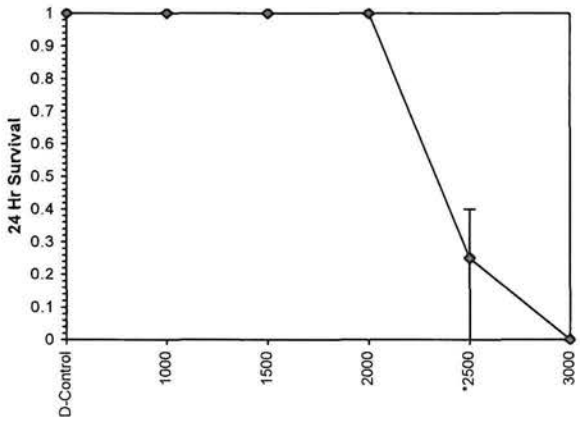
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 2000 | 2500 | 2236.07 | |
| Treatments vs D-Control | | | | |

**Trimmed Spearman-Karber**

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 2352.32 | 2261.76 | 2446.51 |
| 5.0% | 2343.17 | 2244.68 | 2445.98 |
| 10.0% | 2334.93 | 2229.9 | 2444.9 |
| 20.0% | 2322.88 | 2211.45 | 2439.93 |
| Auto-0.0% | 2352.32 | 2261.76 | 2446.51 |




**Dose-Response Plot**



# Statistical Analyses

**Acute Daphnid Test-48 Hr Survival**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 6/3/2025 | Test ID: | CdNaClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 6/5/2025 | 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 |
| 1000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 1500 | 1.0000 | 1.0000 | 1.0000 | 0.8000 |
| 2000 | 0.6000 | 0.2000 | 0.2000 | 0.4000 |
| 2500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | | | 0 | 20 |
| 1000 | 1.0000 | 1.0000 | 1.3453 | 1.3453 | 1.3453 | 0.000 | 4 | 18.00 | 10.00 | 0 | 20 |
| 1500 | 0.9500 | 0.9500 | 1.2857 | 1.1071 | 1.3453 | 9.261 | 4 | 16.00 | 10.00 | 1 | 20 |
| *2000 | 0.3500 | 0.3500 | 0.6245 | 0.4636 | 0.8861 | 32.527 | 4 | 10.00 | 10.00 | 13 | 20 |
| 2500 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |
| 3000 | 0.0000 | 0.0000 | 0.2255 | 0.2255 | 0.2255 | 0.000 | 4 | | | 20 | 20 |

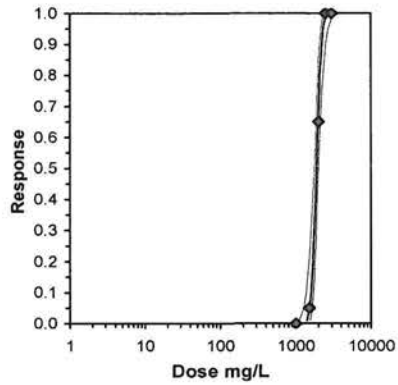
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.82752 | 0.844 | 0.30799 | 2.03482 |
| Equality of variance cannot be confirmed | | | | |

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

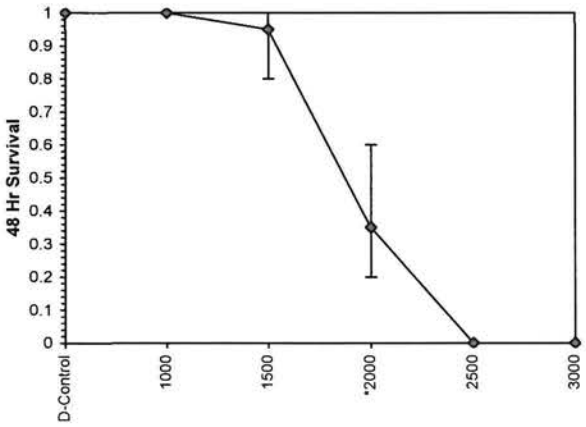
**Maximum Likelihood-Probit**

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 18.0609 | 3.75777 | 10.6957 | 25.4261 | 0 | 0.45678 | 7.81472 | 0.92828 | 3.27413 | 0.05537 | 4 |
| Intercept | -54.134 | 12.342 | -78.324 | -29.943 | | | | | | | |
| TSCR | | | | | | | | | | | |

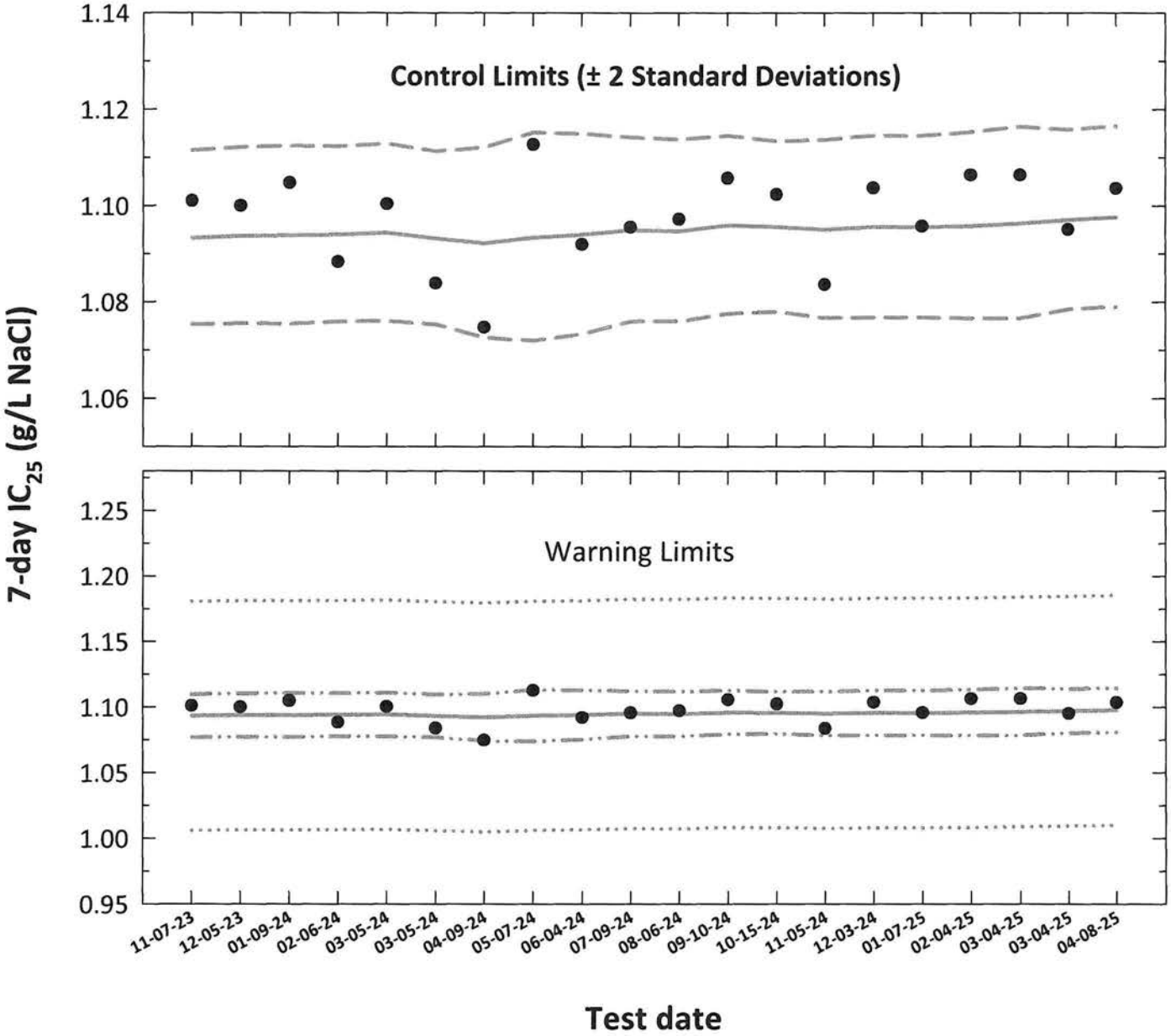
| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 1397.41 | 1107.07 | 1553.26 |
| EC05 | 3.355 | 1524.26 | 1276.37 | 1659.45 |
| EC10 | 3.718 | 1596.52 | 1375.17 | 1721.23 |
| EC15 | 3.964 | 1647.2 | 1444.87 | 1765.73 |
| EC20 | 4.158 | 1688.62 | 1501.71 | 1803.2 |
| EC25 | 4.326 | 1724.99 | 1551.22 | 1837.19 |
| EC40 | 4.747 | 1820.13 | 1676.75 | 1933.3 |
| EC50 | 5.000 | 1879.88 | 1750.48 | 2001.06 |
| EC60 | 5.253 | 1941.59 | 1820.9 | 2078.65 |
| EC75 | 5.674 | 2048.69 | 1928.83 | 2232.1 |
| EC80 | 5.842 | 2092.81 | 1968.84 | 2301.42 |
| EC85 | 6.036 | 2145.44 | 2014.02 | 2387.91 |
| EC90 | 6.282 | 2213.54 | 2069.33 | 2505.02 |
| EC95 | 6.645 | 2318.48 | 2149.66 | 2694.79 |
| EC99 | 7.326 | 2528.93 | 2300.11 | 3102.18 |




**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 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 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 |
| 10 | 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 |
| 11 | 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 |
| 12 | 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 |
| 13 | 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 |
| 14 | 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 |
| 15 | 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 |
| 16 | 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 |
| 17 | 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 |
| 18 | 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 |
| 19 | 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 |
| 20 | 04-08-25 | 1.1036 | 0.0428 | 0.0405 | 0.0037 | 1.0976 | 1.0791 | 1.1165 | 1.0807 | 1.1145 | 1.0098 | 1.1854 |

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

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

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

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

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

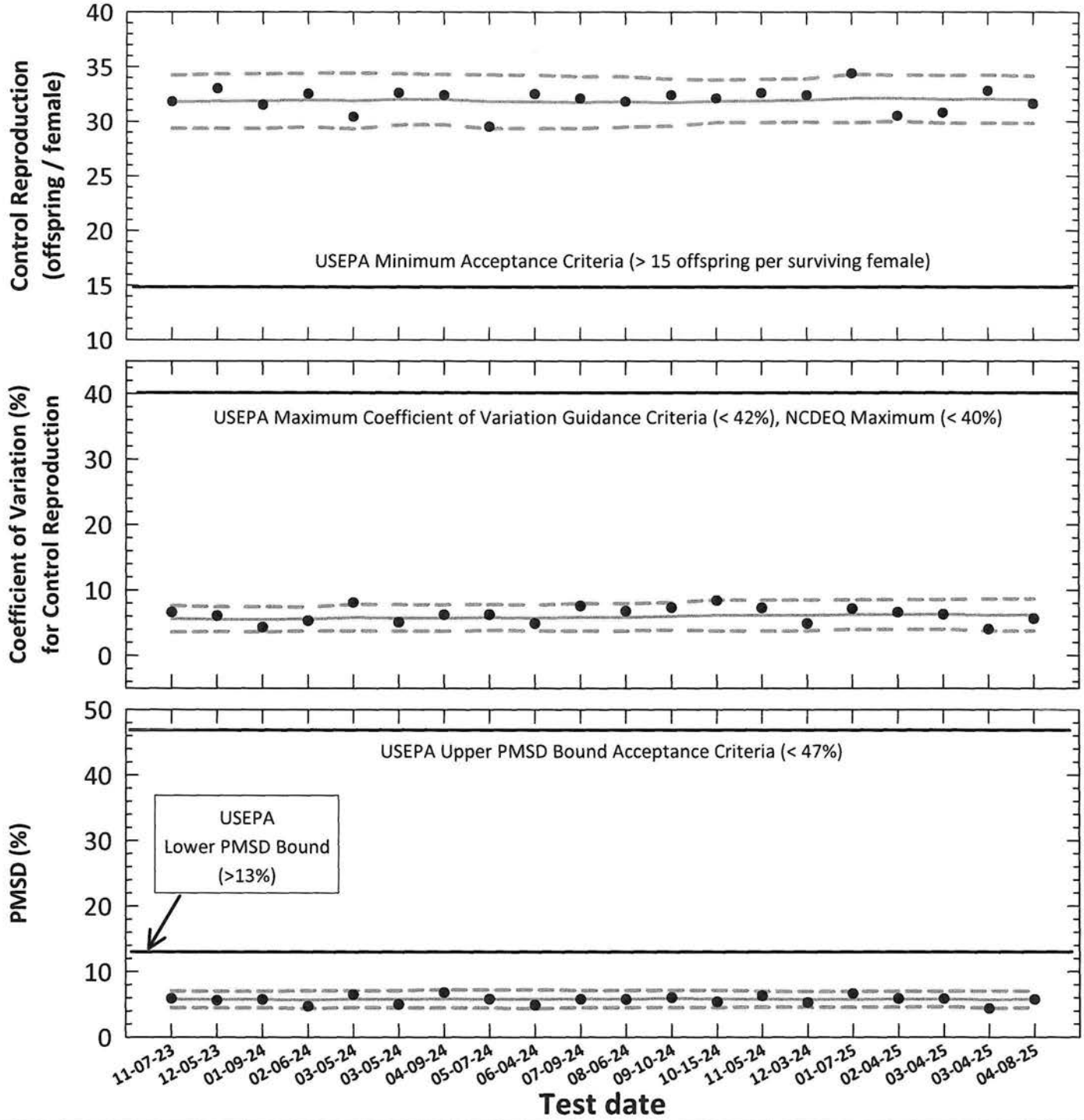
$S_{A.10}$ = Standard deviation corresponding to the $10^{th}$ percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

**CV** = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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




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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

| Test number | Test date | ToxCal Determination | | | | | Control Reproduction (offspring/female) | | | Control Reproduction CV (%) | | | Test PMSD (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control Survival (%) | Control Reproduction Mean (offspring/female) | Control Reproduction CV (%) | Test MSD | Test PMSD (%) | CT | 95% Confidence Interval CT - 2S | 95% Confidence Interval CT + 2S | CT | 95% Confidence Interval CT - 2S | 95% Confidence Interval CT + 2S | CT | 95% Confidence Interval CT - 2S | 95% Confidence Interval CT + 2S |
| 1 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 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 |
| 10 | 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 |
| 11 | 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 |
| 12 | 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 |
| 13 | 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 |
| 14 | 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 |
| 15 | 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 |
| 16 | 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 |
| 17 | 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 |
| 18 | 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 |
| 19 | 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 |
| 20 | 04-08-25 | 100 | 31.6 | 5.6 | 1.806 | 5.7 | 32.0 | 29.8 | 34.1 | 6.2 | 3.8 | 8.7 | 5.7 | 4.4 | 7.0 |

*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 #: 310**

| ***Dilution preparation information:*** | | | | | | ***Comments:*** |
|---|---|---|---|---|---|---|
| NaCl Stock INSS number: | | INSS 2368 | | | | |
| 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 04-08-25 | | | | | | | | | |
| Date and times organisms were born between: | 04-08-25 ~~0530~~ 0530 TO 0800 | | | | | | | | | |
| Culture board: | 04-01-25 A | | | | | | | | | |
| Replicate number: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Culture board cup number: | 2 | 3 | 4 | 8 | 10 | 13 | 17 | 22 | 23 | 24 |
| Transfer vessel information: | pH (S.U.): 7.94 Temperature (°C): 24.9 | | | | | | | | | |
| Average transfer volume (mL): | < 0.25 mL | | | | | | | | | |

***Test randomization and location:***

| | |
|---|---|
| Randomizing template color: | Blue |
| Incubator number and shelf location: | 2B1 |

***Daily renewal:***

| Day | Date | Test initiation and feeding, renewal and feeding, or termination time | *Feeding Batches *Selenastrum* | *Feeding Batches YWT | MHSW batch used | Analyst |
|---|---|---|---|---|---|---|
| 0 | 04-08-25 | 0830 | 03-27-25 | 03-21-25 | 04-02-25B | [illegible] |
| 1 | 04-09-25 | 0757 | ↓ | ↓ | ↓ | [illegible] |
| 2 | 04-10-25 | 0745 | ↓ | ↓ | 04-02-25D | [illegible] |
| 3 | 04-11-25 | 0733 | ↓ | ↓ | ↓ | [illegible] |
| 4 | 04-12-25 | 0800 | ↓ | ↓ | 04-10-25A | [illegible] |
| 5 | 04-13-25 | 0741 | ↓ | ↓ | ↓ | [illegible] |
| 6 | 04-14-25 | 0740 | ↓ | ↓ | ↓ | [illegible] |
| 7 | 04-15-25 | 0631 | | | | [illegible] |

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

***Chemical analyses:***

| Parameter | Reporting Limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved Oxygen (D.O.) | 1.0 mg/L | SM 4500-O G-2021 | YSI Model 52CE | 08A100271 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2021 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2021 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2021 | Not applicable | Not applicable |
| Temperature | 0.1°C | SM 2560B-2010 | Digital Thermometer | 13066468S |

| ***Control information:*** | | Acceptance criteria | ***Summary of test endpoints:*** | |
|---|---|---|---|---|
| % of Male Adults: | 0% | ≤ 20% | **7-day $LC_{50}$** (mg/L NaCl) | >1400 |
| % Adults having 3rd Broods: | 100% | ≥ 80% | **NOEC** (mg/L NaCl) | 1000 |
| % Mortality: | 0% | ≤ 20% | **LOEC** (mg/L NaCl) | 1200 |
| Mean Offspring/Female: | 31.6 | ≥ 15.0 offspring/female | **ChV** (mg/L NaCl) | 1095.5 |
| % CV: | 5.6% | < 40.0 % | **$IC_{25}$** (mg/L NaCl) | 1103.6 |

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 310**

**CONTROL** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 5 | 4 | 6 | 4 | 4 | 5 | 5 | 5 | 6 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 11 | 12 | 12 | 10 | 13 | 10 | 12 | 12 | 12 | 11 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 14 | 15 | 14 | 18 | 15 | 15 | 13 | 16 | 15 | 17 |
| Total young produced | | 30 | 32 | 30 | 34 | 32 | 29 | 30 | 33 | 32 | 34 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |
| X for 3rd Broods | | X | X | X | X | X | X | X | X | X | X |

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

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 31.6 |

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

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 5 | 6 | 4 | 5 | 5 | 5 | 6 | 5 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 12 | 13 | 11 | 13 | 10 | 10 | 12 | 12 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 15 | 18 | 15 | 16 | 15 | 15 | 17 | 13 | 19 | 14 |
| Total young produced | | 31 | 35 | 34 | 31 | 33 | 30 | 32 | 31 | 36 | 31 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

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

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 32.4 |
| % Reduction from Control: | -2.5% |

Environmental Testing Solutions, Inc.

**Species: *Ceriodaphnia dubia*** **CdNaClCR #: 310**

**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 | 5 | 6 | 4 | 6 | 4 | 4 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 9 | 12 | 10 | 12 | 12 | 13 | 12 | 12 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 14 | 18 | 16 | 17 | 16 | 16 | 19 | 15 | 15 | 15 |
| Total young produced | | 31 | 31 | 32 | 32 | 34 | 32 | 38 | 31 | 31 | 31 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

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

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 32.3 |
| % Reduction from Control: | -2.2% |

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

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 4 | 5 | 3 | 5 | 5 | 5 | 6 | 4 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 11 | 12 | 12 | 11 | 12 | 13 | 11 | 11 | 13 | 11 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 14 | 14 | 16 | 13 | 14 | 16 | 14 | 15 | 13 | 13 |
| Total young produced | | 30 | 30 | 33 | 27 | 31 | 34 | 30 | 32 | 30 | 28 |
| 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: | 30.5 |
| % Reduction from Control: | 3.5% |

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 310**

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

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 3 | 3 | 4 | 3 | 5 | 3 | 4 | 4 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 9 | 5 | 7 | 9 | 6 | 8 | 5 | 9 | 9 | 7 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 5 | 9 | 10 | 4 | 11 | 4 | 9 | 5 | 5 | 7 |
| Total young produced | | 19 | 17 | 20 | 17 | 20 | 17 | 17 | 18 | 18 | 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: | 18.1 |
| % Reduction from Control: | 42.7% |

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

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

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

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 2.3 |
| % Reduction from Control: | 92.7% |

Environmental Testing Solutions, Inc.

# Verification of *Ceriodaphnia* Reproduction Totals

## Control

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 5 | 4 | 6 | 4 | 4 | 5 | 5 | 5 | 6 | 49 |
| 5 | 11 | 12 | 12 | 10 | 13 | 10 | 12 | 12 | 12 | 11 | 115 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 14 | 15 | 14 | 18 | 15 | 15 | 13 | 16 | 15 | 17 | 152 |
| **Total** | **30** | **32** | **30** | **34** | **32** | **29** | **30** | **33** | **32** | **34** | **316** |

## 1000 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 4 | 5 | 3 | 5 | 5 | 5 | 6 | 4 | 4 | 46 |
| 5 | 11 | 12 | 12 | 11 | 12 | 13 | 11 | 11 | 13 | 11 | 117 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 14 | 14 | 16 | 13 | 14 | 16 | 14 | 15 | 13 | 13 | 142 |
| **Total** | **30** | **30** | **33** | **27** | **31** | **34** | **30** | **32** | **30** | **28** | **305** |

## 600 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 4 | 5 | 6 | 4 | 5 | 5 | 5 | 6 | 5 | 5 | 50 |
| 5 | 12 | 12 | 13 | 11 | 13 | 10 | 10 | 12 | 12 | 12 | 117 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 15 | 18 | 15 | 16 | 15 | 15 | 17 | 13 | 19 | 14 | 157 |
| **Total** | **31** | **35** | **34** | **31** | **33** | **30** | **32** | **31** | **36** | **31** | **324** |

## 1200 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 3 | 3 | 4 | 3 | 5 | 3 | 4 | 4 | 4 | 38 |
| 5 | 9 | 5 | 7 | 9 | 6 | 8 | 5 | 9 | 9 | 7 | 74 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 5 | 9 | 10 | 4 | 11 | 4 | 9 | 5 | 5 | 7 | 69 |
| **Total** | **19** | **17** | **20** | **17** | **20** | **17** | **17** | **18** | **18** | **18** | **181** |

## 800 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 4 | 4 | 5 | 6 | 4 | 6 | 4 | 4 | 4 | 46 |
| 5 | 12 | 9 | 12 | 10 | 12 | 12 | 13 | 12 | 12 | 12 | 116 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 14 | 18 | 16 | 17 | 16 | 16 | 19 | 15 | 15 | 15 | 161 |
| **Total** | **31** | **31** | **32** | **32** | **34** | **32** | **38** | **31** | **31** | **31** | **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 | 2 | 2 | 2 | 1 | 1 | 1 | 3 | 1 | 2 | 3 | 18 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 5 |
| **Total** | **3** | **2** | **2** | **1** | **3** | **1** | **3** | **3** | **2** | **3** | **23** |

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:** 310

**Test dates:** April 08-15, 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 | 32 | 30 | 34 | 32 | 29 | 30 | 33 | 32 | 34 | **100** | **31.6** | **5.6** | **Not applicable** |
| **600** | 31 | 35 | 34 | 31 | 33 | 30 | 32 | 31 | 36 | 31 | **100** | **32.4** | **6.2** | **-2.5** |
| **800** | 31 | 31 | 32 | 32 | 34 | 32 | 38 | 31 | 31 | 31 | **100** | **32.3** | **6.9** | **-2.2** |
| **1000** | 30 | 30 | 33 | 27 | 31 | 34 | 30 | 32 | 30 | 28 | **100** | **30.5** | **7.0** | **3.5** |
| **1200** | 19 | 17 | 20 | 17 | 20 | 17 | 17 | 18 | 18 | 18 | **100** | **18.1** | **6.6** | **42.7** |
| **1400** | 3 | 2 | 2 | 1 | 3 | 1 | 3 | 3 | 2 | 3 | **100** | **2.3** | **35.8** | **92.7** |

**Dunnett's MSD value:** 1.806

**PMSD:** 5.7

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

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

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

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

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

# Statistical Analyses

Entered and Reviewed by Jim Sumner

## Ceriodaphnia Survival and Reproduction Test-Reproduction

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 4/8/2025 | Test ID: | CdNaClCR | Sample ID: | REF-Ref Toxicant |
| End Date: | 4/15/2025 | 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 | 32.000 | 30.000 | 34.000 | 32.000 | 29.000 | 30.000 | 33.000 | 32.000 | 34.000 |
| 600 | 31.000 | 35.000 | 34.000 | 31.000 | 33.000 | 30.000 | 32.000 | 31.000 | 36.000 | 31.000 |
| 800 | 31.000 | 31.000 | 32.000 | 32.000 | 34.000 | 32.000 | 38.000 | 31.000 | 31.000 | 31.000 |
| 1000 | 30.000 | 30.000 | 33.000 | 27.000 | 31.000 | 34.000 | 30.000 | 32.000 | 30.000 | 28.000 |
| 1200 | 19.000 | 17.000 | 20.000 | 17.000 | 20.000 | 17.000 | 17.000 | 18.000 | 18.000 | 18.000 |
| 1400 | 3.000 | 2.000 | 2.000 | 1.000 | 3.000 | 1.000 | 3.000 | 3.000 | 2.000 | 3.000 |

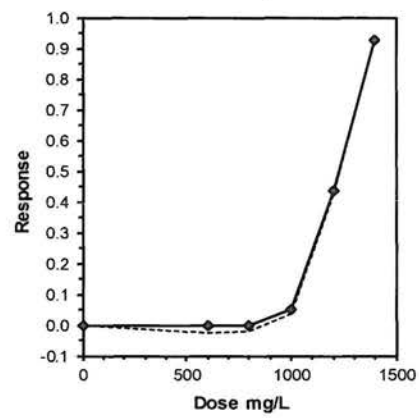
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| D-Control | 31.600 | 1.0000 | 31.600 | 29.000 | 34.000 | 5.621 | 10 | | | | 32.100 | 1.0000 |
| 600 | 32.400 | 1.0253 | 32.400 | 30.000 | 36.000 | 6.207 | 10 | -1.013 | 2.287 | 1.806 | 32.100 | 1.0000 |
| 800 | 32.300 | 1.0222 | 32.300 | 31.000 | 38.000 | 6.853 | 10 | -0.886 | 2.287 | 1.806 | 32.100 | 1.0000 |
| 1000 | 30.500 | 0.9652 | 30.500 | 27.000 | 34.000 | 6.955 | 10 | 1.393 | 2.287 | 1.806 | 30.500 | 0.9502 |
| *1200 | 18.100 | 0.5728 | 18.100 | 17.000 | 20.000 | 6.614 | 10 | 17.094 | 2.287 | 1.806 | 18.100 | 0.5639 |
| *1400 | 2.300 | 0.0728 | 2.300 | 1.000 | 3.000 | 35.794 | 10 | 37.100 | 2.287 | 1.806 | 2.300 | 0.0717 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates normal distribution (p > 0.01) | 0.96973 | 1.035 | 0.84648 | 1.2311 |
| Bartlett's Test indicates equal variances (p = 0.06) | 10.4671 | 15.0863 | | |

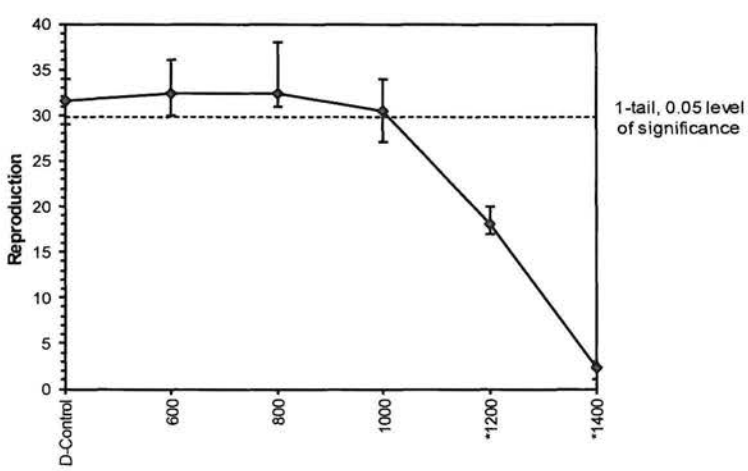
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 1000 | 1200 | 1095.45 | | 1.80589 | 0.05715 | 1486.91 | 3.11852 | 2.3E-43 | 5, 54 |
| Treatments vs D-Control | | | | | | | | | | |

### Linear Interpolation (200 Resamples)

| Point | mg/L | SD | 95% CL | | Skew |
|---|---|---|---|---|---|
| IC05 | 1000.08 | 40.1913 | 886.182 | 1021.33 | -0.7820 |
| IC10 | 1025.97 | 10.8886 | 1003.8 | 1044.85 | -0.9909 |
| IC15 | 1051.85 | 9.10647 | 1032.73 | 1067.76 | -0.2858 |
| IC20 | 1077.74 | 8.02482 | 1060.48 | 1091.96 | -0.1998 |
| IC25 | 1103.63 | 7.13611 | 1088.86 | 1116.3 | -0.0787 |
| IC40 | 1181.29 | 6.37237 | 1169.66 | 1195.2 | 0.4288 |
| IC50 | 1225.95 | 4.48151 | 1217.33 | 1235.32 | 0.2720 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 310**

## *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.

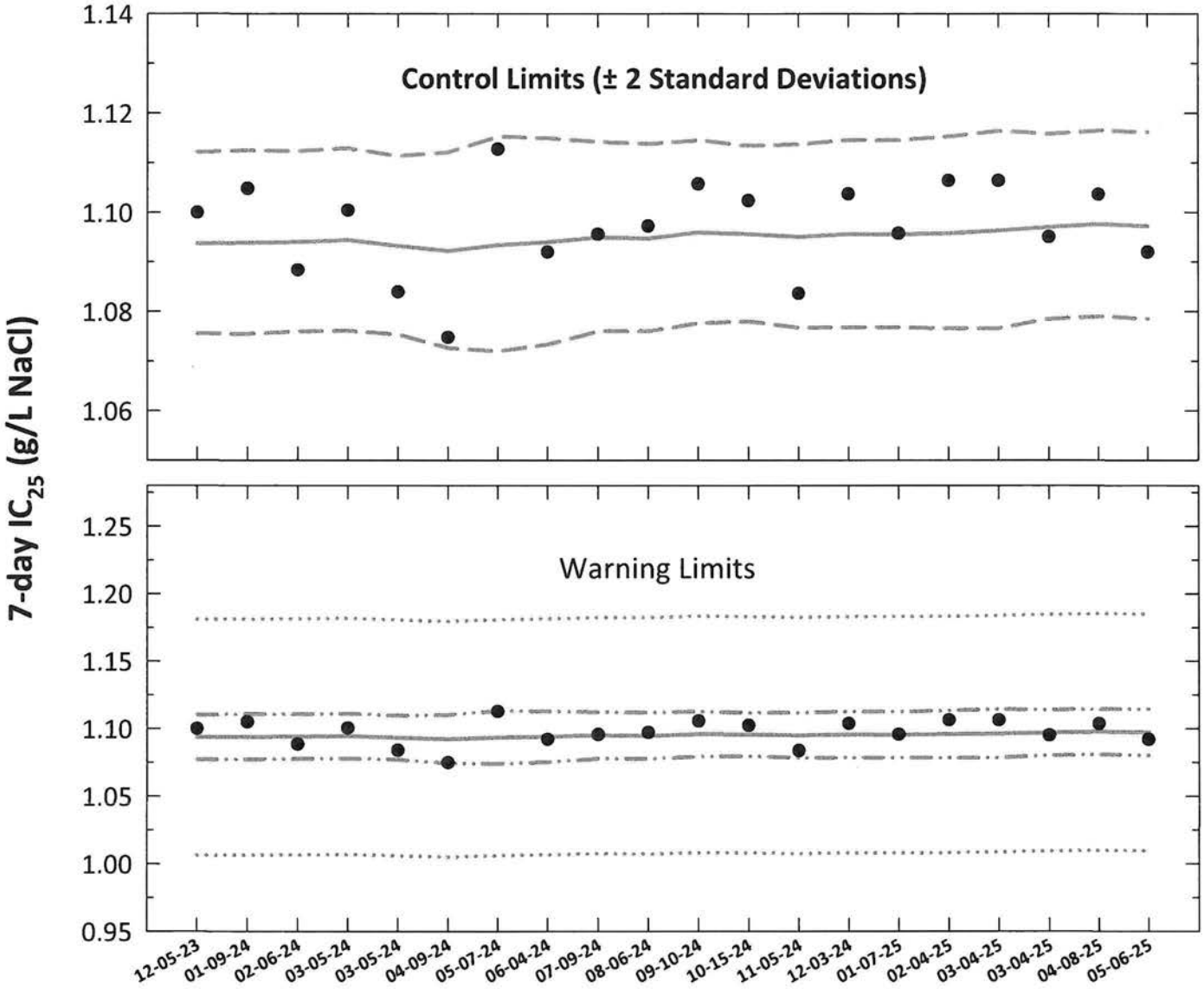
| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | Em | Em M | Em M | Em M | Em M | M |
| Concentration | Parameter | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.89 | 7.89 | 7.84 | 7.90 | 7.86 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 8.1 | 8.2 | 8.2 | 7.8 |
| | Conductivity (µmhos/cm) | 297 | | 312 | | 306 | |
| | Alkalinity (mg $CaCO_3$/L) | 59 | | | | 59 | |
| | Hardness (mg $CaCO_3$/L) | 89 | | | 04-15-25 M | 89 | |
| | Temperature (°C) | 24.9 | 25.2 | 24.8 | 25.2 | 24.8 | 25.3 |
| 600 mg NaCl/L | pH (S.U.) | 7.98 | 7.89 | 7.92 | 7.87 | 7.92 | 7.81 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.1 | 8.1 | 8.2 | 7.7 |
| | Conductivity (µmhos/cm) | 1410 | | 1410 | | 1370 | |
| | Temperature (°C) | 25.0 | 25.0 | 24.9 | 25.0 | 24.9 | 25.2 |
| 800 mg NaCl/L | pH (S.U.) | 7.98 | 7.89 | 7.91 | 7.87 | 7.95 | 7.82 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.1 | 8.1 | 8.2 | 7.7 |
| | Conductivity (µmhos/cm) | 1760 | | 1800 | | 1810 | |
| | Temperature (°C) | 25.0 | 25.0 | 24.9 | 25.3 | 24.9 | 25.2 |
| 1000 mg NaCl/L | pH (S.U.) | 7.99 | 7.89 | 7.94 | 7.87 | 7.96 | 7.82 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.1 | 8.1 | 8.2 | 7.7 |
| | Conductivity (µmhos/cm) | 2120 | | 2180 | | 2170 | |
| | Temperature (°C) | 24.9 | 24.9 | 24.9 | 25.3 | 24.8 | 25.0 |
| 1200 mg NaCl/L | pH (S.U.) | 8.00 | 7.88 | 7.95 | 7.89 | 7.95 | 7.83 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.1 | 8.1 | 8.2 | 7.7 |
| | Conductivity (µmhos/cm) | 2490 | | 2530 | | 2540 | |
| | Temperature (°C) | 24.9 | 24.9 | 24.9 | 25.1 | 24.9 | 25.0 |
| 1400 mg NaCl/L | pH (S.U.) | 8.00 | 7.87 | 7.92 | 7.88 | 7.96 | 7.83 |
| | Dissolved oxygen (mg/L) | 8.4 | 7.9 | 8.0 | 8.1 | 8.2 | 7.6 |
| | Conductivity (µmhos/cm) | 2920 | | 2890 | | 2880 | |
| | Temperature (°C) | 24.9 | 24.9 | 25.0 | 24.9 | 24.9 | 24.9 |
| | | Initial | Final | Initial | Final | Initial | Final |

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 310**

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | N | BSL | BSL | BSL | BSL | EM | EM | N |
| Concentration | Parameter | | | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.89 | 7.97 | 7.94 | 7.89 | 7.97 | 7.89 | 7.88 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | BSL 04.12.25 ~~294~~ 8.2 | 7.9 | 8.4 | 8.1 | 8.2 | 7.7 |
| | Conductivity (µmhos/cm) | 299 | | BSL 04.12.25 ~~8~~ 294 | | 298 | | 316 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 58 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | N 04-15-25 N | 63 | | | | | N 04-15-25 N |
| | Temperature (°C) | 24.8 | 25.1 | 24.9 | 25.2 | 24.9 | 25.2 | 24.8 | 25.1 |
| 600 mg NaCl/L | pH (S.U.) | ~~7.89~~ 7.96 | 7.99 | 7.99 | 7.92 | 8.08 | 7.88 | 7.92 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 8.1 | 7.9 | 8.0 | 8.1 | 8.2 | 7.7 |
| | Conductivity (µmhos/cm) | 1370 | | 1350 | | 1380 | | 1420 | |
| | Temperature (°C) | 24.9 | 24.9 | 25.0 | 25.2 | 25.0 | 25.0 | 24.9 | 25.2 |
| 800 mg NaCl/L | pH (S.U.) | 8.00 | 8.00 | 8.03 | 7.92 | 8.05 | 7.88 | 7.94 | 7.90 |
| | Dissolved oxygen (mg/L) | 8.1 | 8.0 | 8.2 | 7.9 | 8.1 | 8.1 | 8.2 | 7.6 |
| | Conductivity (µmhos/cm) | 1710 | | 1740 | | 1780 | | 1840 | |
| | Temperature (°C) | 24.9 | 25.2 | 25.0 | 25.2 | 25.0 | 25.0 | 25.0 | 25.0 |
| 1000 mg NaCl/L | pH (S.U.) | 8.00 | 7.98 | 8.03 | 7.93 | 8.04 | 7.89 | 7.96 | 7.90 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.9 | 8.2 | 7.9 | 8.1 | 8.1 | 8.2 | 7.7 |
| | Conductivity (µmhos/cm) | 2110 | | 2100 | | 2140 | | 2220 | |
| | Temperature (°C) | 25.0 | 25.2 | 24.9 | 25.2 | 25.0 | 24.9 | 25.0 | 24.8 |
| 1200 mg NaCl/L | pH (S.U.) | 8.01 | 7.98 | 8.03 | 7.92 | 8.04 | 7.91 | 7.95 | 7.89 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.9 | 8.2 | 7.9 | 8.1 | 8.1 | 8.2 | 7.7 |
| | Conductivity (µmhos/cm) | 2450 | | 2420 | | 2510 | | 2570 | |
| | Temperature (°C) | 25.0 | 24.8 | 24.9 | 25.0 | 25.0 | 25.2 | 25.0 | 24.8 |
| 1400 mg NaCl/L | pH (S.U.) | 8.01 | 7.99 | 8.04 | 7.91 | 8.04 | 7.89 | 7.94 | 7.90 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.9 | 8.1 | 7.9 | 8.1 | 8.1 | 8.2 | 7.7 |
| | Conductivity (µmhos/cm) | 2860 | | 2800 | | 2910 | | 2950 | |
| | Temperature (°C) | 25.0 | 24.8 | 24.9 | 25.0 | 25.0 | 25.1 | 25.0 | 24.8 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |

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




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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

| Test number | Test date | 7-day $IC_{25}$ ToxCal Determination (g/L NaCl) | $Log_{10}$ Conversion | | | Anti-logarithmic Values (g/L NaCl) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 7-day $IC_{25}$ | CT | S | CT | Control Limits | | Laboratory Calculated CV Warning Limits | | 10th Percentile CV Warning Limits | |
| | | | | | | | CT - 2S | CT + 2S | CT - 2CV | CT + 2CV | CT - $S_{A.10}$ | CT + $S_{A.10}$ |
| 1 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 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 |
| 10 | 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 |
| 11 | 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 |
| 12 | 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 |
| 13 | 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 |
| 14 | 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 |
| 15 | 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 |
| 16 | 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 |
| 17 | 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 |
| 18 | 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 |
| 19 | 04-08-25 | 1.1036 | 0.0428 | 0.0405 | 0.0037 | 1.0976 | 1.0791 | 1.1165 | 1.0807 | 1.1145 | 1.0098 | 1.1854 |
| 20 | 05-06-25 | 1.0920 | 0.0382 | 0.0403 | 0.0037 | 1.0972 | 1.0785 | 1.1162 | 1.0802 | 1.1142 | 1.0094 | 1.1850 |

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

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

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

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

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

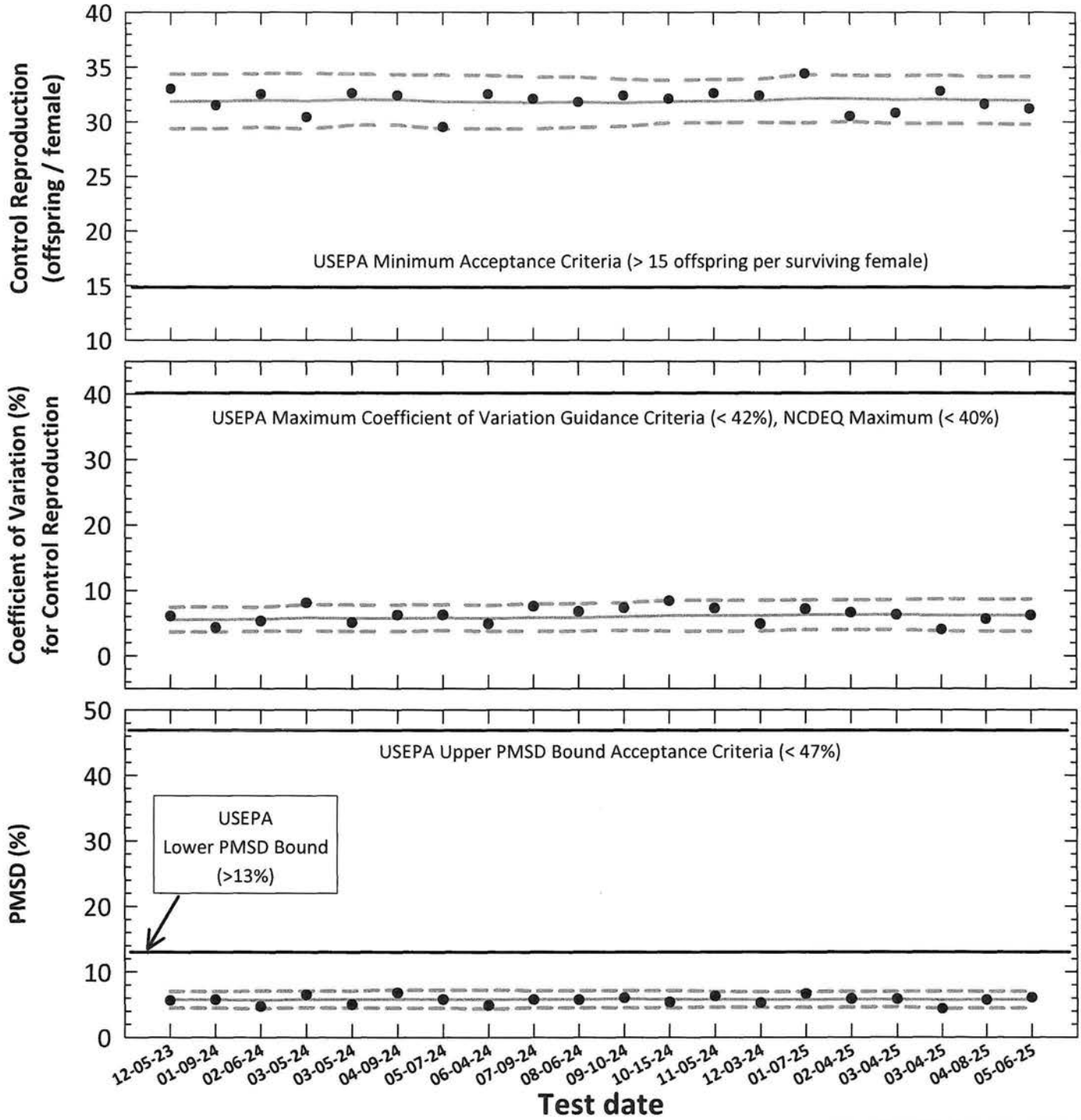
$S_{A.10}$ = Standard deviation corresponding to the $10^{th}$ percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

**CV** = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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




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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

| Test number | Test date | ToxCal Determination | | | | | Control Reproduction (offspring/female) | | | Control Reproduction CV (%) | | | Test PMSD (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | 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 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 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 |
| 10 | 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 |
| 11 | 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 |
| 12 | 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 |
| 13 | 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 |
| 14 | 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 |
| 15 | 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 |
| 16 | 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 |
| 17 | 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 |
| 18 | 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 |
| 19 | 04-08-25 | 100 | 31.6 | 5.6 | 1.806 | 5.7 | 32.0 | 29.8 | 34.1 | 6.2 | 3.8 | 8.7 | 5.7 | 4.4 | 7.0 |
| 20 | 05-06-25 | 100 | 31.2 | 6.2 | 1.889 | 6.1 | 32.0 | 29.8 | 34.1 | 6.2 | 3.8 | 8.6 | 5.7 | 4.4 | 7.0 |

*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 #: 311**

| ***Dilution preparation information:*** | | | | | | ***Comments:*** |
|---|---|---|---|---|---|---|
| NaCl Stock INSS number: | | INSS 7375 | | | | |
| 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: | 05.06.25 0530 TO 0750 | | | | | | | | | |
| Culture board: | 04.29.25 A | | | | | | | | | |
| Replicate number: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Culture board cup number: | 1 | 2 | 5 | 10 | 11 | 12 | 13 | 17 | 20 | 21 |
| Transfer vessel information: | pH (S.U.): 8.03 Temperature (°C): 25.0 | | | | | | | | | |
| Average transfer volume (mL): | < 0.25 mL | | | | | | | | | |

***Test randomization and location:***

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

***Daily renewal:***

| Day | Date | Test initiation and feeding, renewal and feeding, or termination time | *Feeding Batches *Selenastrum* | *Feeding Batches YWT | MHSW batch used | Analyst |
|---|---|---|---|---|---|---|
| 0 | 05-06-25 | 0810 | 04.25.25 | 04.25.25 | 04.30.25 B | M |
| 1 | 05-07-25 | 0742 | ↓ | ↓ | ↓ | M |
| 2 | 05-08-25 | 0630 | ↓ | ↓ | 04.30.25 C | M |
| 3 | 05-09-25 | 0620 | ↓ | ↓ | ↓ | M |
| 4 | 05-10-25 | 0730 | ↓ | ↓ | 05.08.25 A | M |
| 5 | 05-11-25 | 0700 | ↓ | ↓ | ↓ | M |
| 6 | 05-12-25 | 0610 | ↓ | ↓ | ↓ | M |
| 7 | 05-13-25 | 0615 | | | | M |

*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: | 31.2 | ≥ 15.0 offspring/female | **ChV** (mg/L NaCl) | ~~1095.5~~ 1095.4 |
| % CV: | 6.27% | < 40.0 % | **$IC_{25}$** (mg/L NaCl) | 1092.0 |

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 311**

**CONTROL** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 5 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 3 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 10 | 10 | 12 | 12 | 9 | 12 | 13 | 12 | 11 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 16 | 13 | 15 | 17 | 17 | 15 | 18 | 15 | 19 | 16 |
| Total young produced | | 30 | 28 | 31 | 33 | 30 | 31 | 34 | 30 | 34 | 31 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |
| X for 3rd Broods | | X | X | X | X | X | X | X | X | X | X |

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

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 31.2 |

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

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 4 | 5 | 5 | 3 | 3 | 3 | 5 | 4 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 11 | 11 | 13 | 12 | 12 | 10 | 12 | 13 | 11 | 13 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 16 | 16 | 17 | 15 | 18 | 14 | 15 | 17 | 16 | 14 |
| Total young produced | | 31 | 31 | 35 | 32 | 33 | 27 | 30 | 35 | 31 | 31 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

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

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 31.6 |
| % Reduction from Control: | -1.3% |

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 311**

**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 | 5 | 5 | 5 | 4 | 4 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 13 | 12 | 12 | 10 | 10 | 12 | 13 | 13 | 10 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 16 | 14 | 19 | 16 | 18 | 15 | 17 | 16 | 14 | 14 |
| Total young produced | | 33 | 31 | 35 | 31 | 33 | 30 | 34 | 33 | 31 | 28 |
| 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: | 31.9 |
| % Reduction from Control: | -2.2% |

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

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 3 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 10 | 11 | 11 | 9 | 13 | 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 | 13 | 14 | 13 | 17 | 14 | 15 | 14 | 14 |
| Total young produced | | 30 | 30 | 28 | 27 | 29 | 31 | 28 | 31 | 30 | 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: | 29.1 |
| % Reduction from Control: | 6.7% |

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 311**

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

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

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

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 17.3 |
| % Reduction from Control: | 44.6% |

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

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

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

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 2.6 |
| % Reduction from Control: | 91.7% |

Environmental Testing Solutions, Inc.

# Verification of *Ceriodaphnia* Reproduction Totals

## Control

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 4 | 5 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 3 | 38 |
| 5 | 10 | 10 | 12 | 12 | 9 | 12 | 13 | 12 | 11 | 12 | 113 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 16 | 13 | 15 | 17 | 17 | 15 | 18 | 15 | 19 | 16 | 161 |
| **Total** | **30** | **28** | **31** | **33** | **30** | **31** | **34** | **30** | **34** | **31** | **312** |

## 1000 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 3 | 39 |
| 5 | 10 | 11 | 11 | 9 | 13 | 10 | 10 | 12 | 12 | 10 | 108 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 15 | 15 | 13 | 14 | 13 | 17 | 14 | 15 | 14 | 14 | 144 |
| **Total** | **30** | **30** | **28** | **27** | **29** | **31** | **28** | **31** | **30** | **27** | **291** |

## 600 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 4 | 4 | 5 | 5 | 3 | 3 | 3 | 5 | 4 | 4 | 40 |
| 5 | 11 | 11 | 13 | 12 | 12 | 10 | 12 | 13 | 11 | 13 | 118 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 16 | 16 | 17 | 15 | 18 | 14 | 15 | 17 | 16 | 14 | 158 |
| **Total** | **31** | **31** | **35** | **32** | **33** | **27** | **30** | **35** | **31** | **31** | **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 | 3 | 3 | 2 | 3 | 4 | 4 | 2 | 3 | 3 | 3 | 30 |
| 5 | 9 | 6 | 6 | 8 | 6 | 10 | 7 | 9 | 8 | 5 | 74 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 5 | 8 | 5 | 7 | 6 | 5 | 10 | 7 | 7 | 9 | 69 |
| **Total** | **17** | **17** | **13** | **18** | **16** | **19** | **19** | **19** | **18** | **17** | **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 | 5 | 5 | 5 | 4 | 4 | 4 | 43 |
| 5 | 12 | 13 | 12 | 12 | 10 | 10 | 12 | 13 | 13 | 10 | 117 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 16 | 14 | 19 | 16 | 18 | 15 | 17 | 16 | 14 | 14 | 159 |
| **Total** | **33** | **31** | **35** | **31** | **33** | **30** | **34** | **33** | **31** | **28** | **319** |

## 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 | 1 | 1 | 3 | 2 | 2 | 2 | 2 | 3 | 2 | 21 |
| 5 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 4 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| **Total** | **4** | **1** | **1** | **3** | **2** | **3** | **3** | **4** | **3** | **2** | **26** |

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:** 311

**Test dates:** May 06-13, 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 | 28 | 31 | 33 | 30 | 31 | 34 | 30 | 34 | 31 | **100** | **31.2** | **6.2** | **Not applicable** |
| **600** | 31 | 31 | 35 | 32 | 33 | 27 | 30 | 35 | 31 | 31 | **100** | **31.6** | **7.5** | **-1.3** |
| **800** | 33 | 31 | 35 | 31 | 33 | 30 | 34 | 33 | 31 | 28 | **100** | **31.9** | **6.5** | **-2.2** |
| **1000** | 30 | 30 | 28 | 27 | 29 | 31 | 28 | 31 | 30 | 27 | **100** | **29.1** | **5.2** | **6.7** |
| **1200** | 17 | 17 | 13 | 18 | 16 | 19 | 19 | 19 | 18 | 17 | **100** | **17.3** | **10.6** | **44.6** |
| **1400** | 4 | 1 | 1 | 3 | 2 | 3 | 3 | 4 | 3 | 2 | **100** | **2.6** | **41.3** | **91.7** |

**Dunnett's MSD value:** 1.889

**PMSD:** 6.1

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: | 5/6/2025 | Test ID: | CdNaCICR | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/13/2025 | 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 | 28.000 | 31.000 | 33.000 | 30.000 | 31.000 | 34.000 | 30.000 | 34.000 | 31.000 |
| 600 | 31.000 | 31.000 | 35.000 | 32.000 | 33.000 | 27.000 | 30.000 | 35.000 | 31.000 | 31.000 |
| 800 | 33.000 | 31.000 | 35.000 | 31.000 | 33.000 | 30.000 | 34.000 | 33.000 | 31.000 | 28.000 |
| 1000 | 30.000 | 30.000 | 28.000 | 27.000 | 29.000 | 31.000 | 28.000 | 31.000 | 30.000 | 27.000 |
| 1200 | 17.000 | 17.000 | 13.000 | 18.000 | 16.000 | 19.000 | 19.000 | 19.000 | 18.000 | 17.000 |
| 1400 | 4.000 | 1.000 | 1.000 | 3.000 | 2.000 | 3.000 | 3.000 | 4.000 | 3.000 | 2.000 |

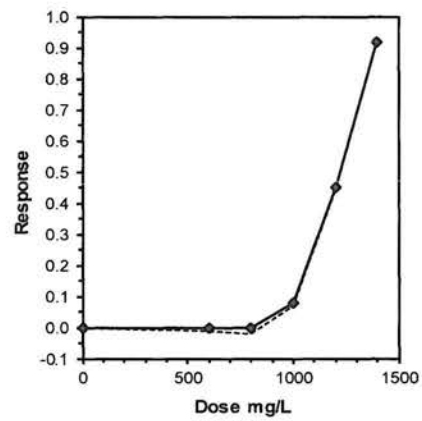
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| D-Control | 31.200 | 1.0000 | 31.200 | 28.000 | 34.000 | 6.193 | 10 | | | | 31.567 | 1.0000 |
| 600 | 31.600 | 1.0128 | 31.600 | 27.000 | 35.000 | 7.489 | 10 | -0.484 | 2.287 | 1.889 | 31.567 | 1.0000 |
| 800 | 31.900 | 1.0224 | 31.900 | 28.000 | 35.000 | 6.517 | 10 | -0.847 | 2.287 | 1.889 | 31.567 | 1.0000 |
| *1000 | 29.100 | 0.9327 | 29.100 | 27.000 | 31.000 | 5.237 | 10 | 2.542 | 2.287 | 1.889 | 29.100 | 0.9219 |
| *1200 | 17.300 | 0.5545 | 17.300 | 13.000 | 19.000 | 10.571 | 10 | 16.824 | 2.287 | 1.889 | 17.300 | 0.5480 |
| *1400 | 2.600 | 0.0833 | 2.600 | 1.000 | 4.000 | 41.345 | 10 | 34.617 | 2.287 | 1.889 | 2.600 | 0.0824 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates normal distribution (p > 0.01) | 0.51775 | 1.035 | -0.346 | 0.29165 |
| Bartlett's Test indicates equal variances (p = 0.33) | 5.75643 | 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 | | 1.88922 | 0.06055 | 1401.71 | 3.41296 | 1.2E-41 | 5, 54 |
| Treatments vs D-Control | | | | | | | | | | |

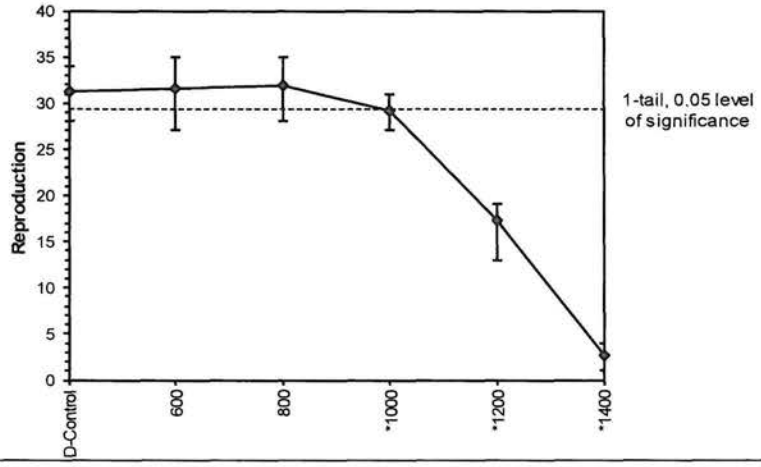
### Linear Interpolation (200 Resamples)

| Point | mg/L | SD | 95% CL | | Skew |
|---|---|---|---|---|---|
| IC05 | 927.973 | 54.3347 | 784.829 | 1001.18 | -3.1130 |
| IC10 | 1011.69 | 17.394 | 961.273 | 1025.4 | -2.0889 |
| IC15 | 1038.45 | 8.92642 | 1017.06 | 1050 | -0.3319 |
| IC20 | 1065.2 | 8.21131 | 1046.58 | 1076.61 | -0.3436 |
| IC25 | 1091.95 | 7.75589 | 1074.78 | 1102.42 | -0.3185 |
| IC40 | 1172.2 | 8.26077 | 1152.38 | 1183.74 | -0.1125 |
| IC50 | 1220.63 | 6.8034 | 1203.35 | 1229.31 | -0.4408 |




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

### Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 311**

## *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.

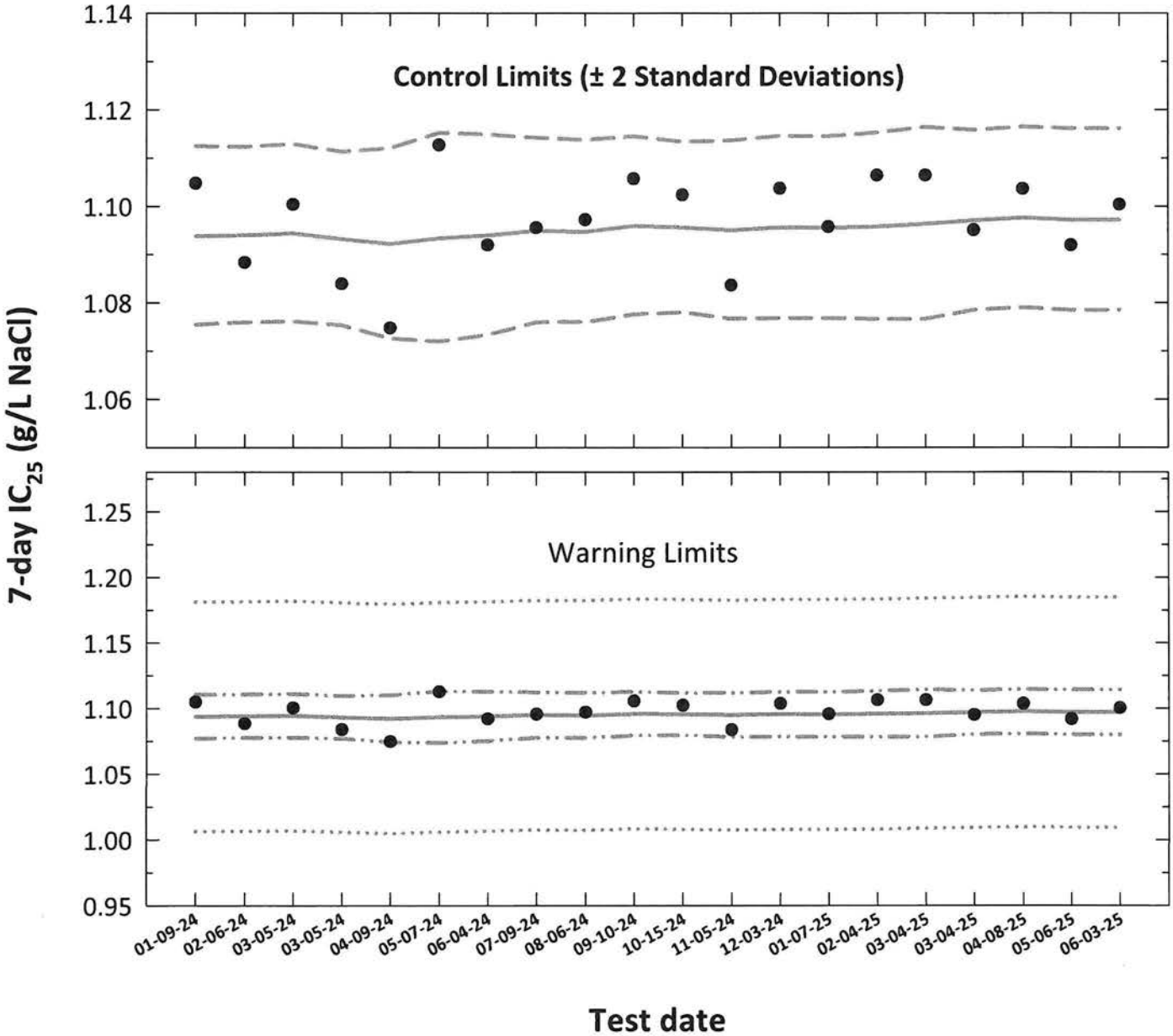
| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | N | EM | EM | EM | EM | N |
| Concentration | Parameter | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 8.13 | 7.86 | 7.80 | 7.84 | 7.81 | 7.88 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.0 | 8.0 | 8.0 | 7.7 |
| | Conductivity (µmhos/cm) | 296 | | 313 | | 300 | |
| | Alkalinity (mg $CaCO_3$/L) | 62 | | | | 60 | |
| | Hardness (mg $CaCO_3$/L) | 88 | | | N 05-14-25 M | 86 | |
| | Temperature (°C) | 24.9 | 25.3 | 24.8 | 25.2 | 24.8 | 24.9 |
| 600 mg NaCl/L | pH (S.U.) | 8.13 | 7.85 | 7.86 | 7.83 | 7.87 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.0 | 8.0 | 8.0 | 7.7 |
| | Conductivity (µmhos/cm) | 1460 | | 1450 | | 1440 | |
| | Temperature (°C) | 25.0 | 25.0 | 24.9 | 25.3 | 24.8 | 25.2 |
| 800 mg NaCl/L | pH (S.U.) | 8.10 | 7.85 | 7.86 | 7.85 | 7.88 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.0 | 8.0 | 8.1 | 7.6 |
| | Conductivity (µmhos/cm) | 1880 | | 1850 | | 1860 | |
| | Temperature (°C) | 24.9 | 25.2 | 24.9 | 25.3 | 24.9 | 25.0 |
| 1000 mg NaCl/L | pH (S.U.) | 8.11 | 7.85 | 7.87 | 7.85 | 7.89 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.0 | 8.0 | 8.1 | 7.6 |
| | Conductivity (µmhos/cm) | 2240 | | 2200 | | 2210 | |
| | Temperature (°C) | 24.9 | 25.2 | 25.0 | 25.0 | 24.9 | 25.0 |
| 1200 mg NaCl/L | pH (S.U.) | 8.11 | 7.85 | 7.88 | 7.83 | 7.89 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.1 | 7.9 | 8.1 | 7.6 |
| | Conductivity (µmhos/cm) | 2600 | | 2580 | | 2570 | |
| | Temperature (°C) | 25.1 | 24.9 | 25.0 | 24.9 | 24.8 | 25.0 |
| 1400 mg NaCl/L | pH (S.U.) | 8.11 | 7.85 | 7.89 | 7.85 | 7.89 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.1 | 7.9 | 8.1 | 7.6 |
| | Conductivity (µmhos/cm) | 3030 | | 2940 | | 2930 | |
| | Temperature (°C) | 25.1 | 25.1 | 25.0 | 24.8 | 24.8 | 25.1 |
| | | Initial | Final | Initial | Final | Initial | Final |

ETS
Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 311**

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | KL | BSL | BSL | BSL | BSL | XL | XL | KL |
| Concentration | Parameter | | | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 8.01 | 7.94 | 7.92 | 7.99 | 7.97 | 7.86 | 8.00 | 7.01 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.1 | ~~7.91~~ 8.4 BSL 05.10.25 | 8.0 | ~~8.2~~ BSL 05.21.25 8.4 | 8.0 | 8.3 | 7.4 |
| | Conductivity (µmhos/cm) | 295 | | 298 | | 296 | | 309 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 59 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | 05-14-25 KL | 88 | | | | 05-14-25 KL | |
| | Temperature (°C) | 24.9 | 25.1 | 24.7 | 25.1 | 24.8 | 25.3 | 24.9 | 25.1 |
| 600 mg NaCl/L | pH (S.U.) | 7.99 | 7.96 | 7.98 | 8.01 | 7.94 | 7.89 | 7.91 | 7.03 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.2 | 8.0 | 8.2 | 8.0 | 8.3 | 7.4 |
| | Conductivity (µmhos/cm) | 1400 | | 1450 | | 1430 | | 1450 | |
| | Temperature (°C) | 25.0 | 24.9 | 24.8 | 25.1 | 24.9 | 25.0 | 25.0 | 24.9 |
| 800 mg NaCl/L | pH (S.U.) | 7.99 | 7.96 | 7.98 | 8.01 | 8.03 | 7.88 | 7.96 | 7.03 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.2 | 8.0 | 8.2 | 8.0 | 8.3 | 7.4 |
| | Conductivity (µmhos/cm) | 1790 | | 1840 | | 1820 | | 1870 | |
| | Temperature (°C) | 24.8 | 24.9 | 24.7 | 25.2 | 25.0 | 25.2 | 25.0 | 24.9 |
| 1000 mg NaCl/L | pH (S.U.) | 7.99 | 7.96 | 7.98 | 8.00 | 8.02 | 7.88 | 7.97 | 7.03 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.2 | 8.0 | 8.2 | 8.0 | 8.3 | 7.4 |
| | Conductivity (µmhos/cm) | 2130 | | 2210 | | 2180 | | 2240 | |
| | Temperature (°C) | 24.8 | 25.0 | 24.9 | 25.2 | 25.0 | 25.2 | 24.9 | 25.3 |
| 1200 mg NaCl/L | pH (S.U.) | 7.99 | 7.95 | 7.98 | 8.00 | 8.02 | 7.90 | 7.97 | 7.04 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.2 | 8.0 | 8.2 | 8.0 | 8.3 | 7.4 |
| | Conductivity (µmhos/cm) | 2490 | | 2530 | | 2540 | | 2550 | |
| | Temperature (°C) | 24.9 | 24.8 | 24.9 | 25.3 | 25.0 | 25.2 | 24.9 | 25.0 |
| 1400 mg NaCl/L | pH (S.U.) | 7.99 | 7.94 | 7.98 | 8.00 | 8.03 | 7.91 | 7.98 | 7.05 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.3 | 8.0 | 8.2 | 8.1 | 8.3 | 7.4 |
| | Conductivity (µmhos/cm) | 2860 | | 2840 | | 2890 | | 2920 | |
| | Temperature (°C) | 24.9 | 24.8 | 24.8 | 25.0 | 25.0 | 25.1 | 24.9 | 25.3 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |

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




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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

| Test number | Test date | 7-day $IC_{25}$ ToxCal Determination (g/L NaCl) | $Log_{10}$ Conversion | | | Anti-logarithmic Values (g/L NaCl) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 7-day $IC_{25}$ | CT | S | CT | Control Limits | | Laboratory Calculated CV Warning Limits | | 10th Percentile CV Warning Limits | |
| | | | | | | | CT - 2S | CT + 2S | CT - 2CV | CT + 2CV | CT - $S_{A.10}$ | CT + $S_{A.10}$ |
| 1 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 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 |
| 10 | 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 |
| 11 | 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 |
| 12 | 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 |
| 13 | 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 |
| 14 | 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 |
| 15 | 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 |
| 16 | 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 |
| 17 | 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 |
| 18 | 04-08-25 | 1.1036 | 0.0428 | 0.0405 | 0.0037 | 1.0976 | 1.0791 | 1.1165 | 1.0807 | 1.1145 | 1.0098 | 1.1854 |
| 19 | 05-06-25 | 1.0920 | 0.0382 | 0.0403 | 0.0037 | 1.0972 | 1.0785 | 1.1162 | 1.0802 | 1.1142 | 1.0094 | 1.1850 |
| 20 | 06-03-25 | 1.1004 | 0.0416 | 0.0403 | 0.0037 | 1.0972 | 1.0785 | 1.1162 | 1.0802 | 1.1142 | 1.0094 | 1.1850 |

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

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

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

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

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

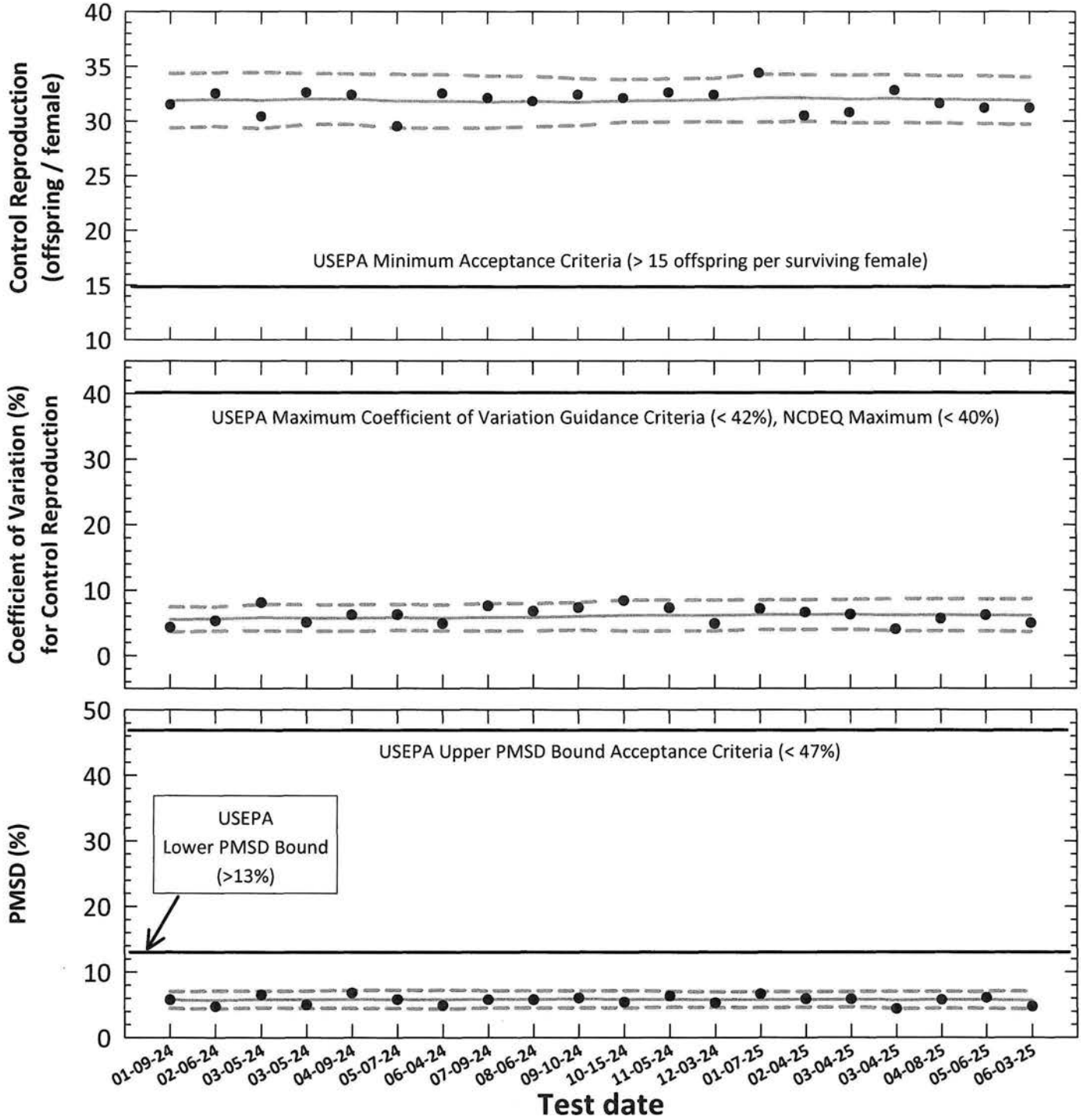
$S_{A.10}$ = Standard deviation corresponding to the $10^{th}$ percentile of CVs reported nationally by USEPA ($S_{A.10}$ = 0.08).

**CV** = Coefficient of variation.

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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




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

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

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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

| Test number | Test date | ToxCal Determination | | | | | Control Reproduction (offspring/female) | | | Control Reproduction CV (%) | | | Test PMSD (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control Survival (%) | Control Reproduction Mean (offspring/female) | Control Reproduction CV (%) | Test MSD | Test PMSD (%) | CT | 95% Confidence Interval CT - 2S | 95% Confidence Interval CT + 2S | CT | 95% Confidence Interval CT - 2S | 95% Confidence Interval CT + 2S | CT | 95% Confidence Interval CT - 2S | 95% Confidence Interval CT + 2S |
| 1 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 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 |
| 10 | 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 |
| 11 | 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 |
| 12 | 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 |
| 13 | 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 |
| 14 | 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 |
| 15 | 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 |
| 16 | 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 |
| 17 | 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 |
| 18 | 04-08-25 | 100 | 31.6 | 5.6 | 1.806 | 5.7 | 32.0 | 29.8 | 34.1 | 6.2 | 3.8 | 8.7 | 5.7 | 4.4 | 7.0 |
| 19 | 05-06-25 | 100 | 31.2 | 6.2 | 1.889 | 6.1 | 32.0 | 29.8 | 34.1 | 6.2 | 3.8 | 8.6 | 5.7 | 4.4 | 7.0 |
| 20 | 06-03-25 | 100 | 31.2 | 5.0 | 1.470 | 4.7 | 31.9 | 29.7 | 34.0 | 6.1 | 3.6 | 8.6 | 5.7 | 4.3 | 7.1 |

*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
JS

Environmental Testing Solutions, Inc.

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

**CdNaClCR #: 312**

| ***Dilution preparation information:*** | | | | | | ***Comments:*** |
|---|---|---|---|---|---|---|
| NaCl Stock INSS number: | | INSS 2393 | | | | |
| 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: | 06-03-25 0530 TO 0750 | | | | | | | | | |
| Culture board: | 05-27-25 A | | | | | | | | | |
| Replicate number: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Culture board cup number: | 1 | 2 | 7 | 8 | 9 | 10 | 17 | 18 | 19 | 22 |
| Transfer vessel information: | pH (S.U.): 7.85 Temperature (°C): 25.0 | | | | | | | | | |
| Average transfer volume (mL): | < 0.25 mL | | | | | | | | | |

### *Test randomization and location:*

| | |
|---|---|
| Randomizing template color: | BLACK |
| Incubator number and shelf location: | 2 B1 |

### *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 | 06-03-25 | 0815 | 05-29-25 | 05-29-25 | 05-28-25 A | [illegible] |
| 1 | 06-04-25 | 0615 | ↓ | ↓ | ↓ | [illegible] |
| 2 | 06-05-25 | 0617 | ↓ | ↓ | 05-28-25 E | [illegible] |
| 3 | 06-06-25 | 0645 | ↓ | ↓ | ↓ | [illegible] |
| 4 | 06-07-25 | 0700 | ↓ | ↓ | 06-02-25 A | [illegible] |
| 5 | 06-08-25 | 0630 | ↓ | ↓ | ↓ | [illegible] |
| 6 | 06-09-25 | 0636 | ↓ | ↓ | ↓ | [illegible] |
| 7 | 06-10-25 | 0715 | | | | [illegible] |

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

### *Chemical analyses:*

| Parameter | Reporting Limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved Oxygen (D.O.) | 1.0 mg/L | SM 4500-O G-2021 | YSI Model 52CE | 08A100271 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2021 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2021 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2021 | Not applicable | Not applicable |
| Temperature | 0.1°C | SM 2560B-2010 | Digital Thermometer | 13066465 |

| ***Control information:*** | | Acceptance criteria | ***Summary of test endpoints:*** | |
|---|---|---|---|---|
| % of Male Adults: | 0% | ≤ 20% | **7-day $LC_{50}$** (mg/L NaCl) | > 1400 |
| % Adults having 3rd Broods: | 100% | ≥ 80% | **NOEC** (mg/L NaCl) | 1000 |
| % Mortality: | 0% | ≤ 20% | **LOEC** (mg/L NaCl) | 1200 |
| Mean Offspring/Female: | 31.2 | ≥ 15.0 offspring/female | **ChV** (mg/L NaCl) | 1095.5 |
| % CV: | 5.0% | < 40.0 % | **$IC_{25}$** (mg/L NaCl) | 1100.4 |

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 312**

**CONTROL** ***Survival and Reproduction Data***

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 4 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 13 | 11 | 12 | 12 | 10 | 9 | 11 | 11 | 11 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 15 | 14 | 16 | 15 | 13 | 18 | 14 | 16 | 15 | 17 |
| Total young produced | | 32 | 31 | 32 | 32 | 29 | 33 | 28 | 32 | 31 | 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: | 31.2 |

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

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 5 | 5 | 6 | 4 | 5 | 5 | 5 | 5 | 6 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 10 | 13 | 10 | 13 | 12 | 12 | 12 | 13 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 15 | 18 | 17 | 17 | 15 | 19 | 16 | 15 | 13 | 16 |
| Total young produced | | 31 | 33 | 35 | 33 | 32 | 36 | 33 | 32 | 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: | 33.0 |
| % Reduction from Control: | -5.8% |

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 312**

**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 | 4 | 4 | 5 | 5 | 5 | 5 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 10 | 10 | 12 | 12 | 10 | 13 | 12 | 10 | 10 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 18 | 18 | 14 | 15 | 18 | 13 | 17 | 19 | 15 | 15 |
| Total young produced | | 33 | 32 | 32 | 31 | 32 | 31 | 34 | 34 | 30 | 32 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

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

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 32.1 |
| % Reduction from Control: | -2.9% |

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

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 6 | 4 | 4 | 3 | 5 | 5 | 5 | 4 | 4 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 9 | 11 | 11 | 12 | 10 | 12 | 10 | 10 | 10 | 11 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 15 | 15 | 16 | 16 | 13 | 17 | 16 | 13 | 16 | 15 |
| Total young produced | | 30 | 30 | 31 | 31 | 28 | 34 | 31 | 27 | 30 | 31 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

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

| ***Concentration:*** | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 30.3 |
| % Reduction from Control: | 2.9% |

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 312**

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

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 3 | 3 | 4 | 2 | 4 | 4 | 3 | 3 | 3 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 8 | 6 | 9 | 10 | 7 | 5 | 8 | 11 | 6 | 10 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 5 | 10 | 4 | 6 | 8 | 10 | 5 | 4 | 9 | 5 |
| Total young produced | | 17 | 19 | 16 | 20 | 17 | 19 | 17 | 18 | 18 | 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: | 17.9 |
| % Reduction from Control: | 42.6% |

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

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

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

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

Environmental Testing Solutions, Inc.

# Verification of *Ceriodaphnia* Reproduction Totals

## Control

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 4 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 4 | 47 |
| 5 | 12 | 13 | 11 | 12 | 12 | 10 | 9 | 11 | 11 | 11 | 112 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 15 | 14 | 16 | 15 | 13 | 18 | 14 | 16 | 15 | 17 | 153 |
| **Total** | **32** | **31** | **32** | **32** | **29** | **33** | **28** | **32** | **31** | **32** | **312** |

## 1000 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 6 | 4 | 4 | 3 | 5 | 5 | 5 | 4 | 4 | 5 | 45 |
| 5 | 9 | 11 | 11 | 12 | 10 | 12 | 10 | 10 | 10 | 11 | 106 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 15 | 15 | 16 | 16 | 13 | 17 | 16 | 13 | 16 | 15 | 152 |
| **Total** | **30** | **30** | **31** | **31** | **28** | **34** | **31** | **27** | **30** | **31** | **303** |

## 600 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 4 | 5 | 5 | 6 | 4 | 5 | 5 | 5 | 5 | 6 | 50 |
| 5 | 12 | 10 | 13 | 10 | 13 | 12 | 12 | 12 | 13 | 12 | 119 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 15 | 18 | 17 | 17 | 15 | 19 | 16 | 15 | 13 | 16 | 161 |
| **Total** | **31** | **33** | **35** | **33** | **32** | **36** | **33** | **32** | **31** | **34** | **330** |

## 1200 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 4 | 3 | 3 | 4 | 2 | 4 | 4 | 3 | 3 | 3 | 33 |
| 5 | 8 | 6 | 9 | 10 | 7 | 5 | 8 | 11 | 6 | 10 | 80 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 5 | 10 | 4 | 6 | 8 | 10 | 5 | 4 | 9 | 5 | 66 |
| **Total** | **17** | **19** | **16** | **20** | **17** | **19** | **17** | **18** | **18** | **18** | **179** |

## 800 mg NaCl/L

| Day | Replicate number | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 5 | 4 | 6 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 48 |
| 5 | 10 | 10 | 12 | 12 | 10 | 13 | 12 | 10 | 10 | 12 | 111 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 18 | 18 | 14 | 15 | 18 | 13 | 17 | 19 | 15 | 15 | 162 |
| **Total** | **33** | **32** | **32** | **31** | **32** | **31** | **34** | **34** | **30** | **32** | **321** |

## 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 | 2 | 2 | 1 | 3 | 17 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **2** | **1** | **3** | **1** | **1** | **1** | **2** | **2** | **1** | **3** | **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:** 312

**Test dates:** June 03-10, 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** | 32 | 31 | 32 | 32 | 29 | 33 | 28 | 32 | 31 | 32 | **100** | **31.2** | **5.0** | **Not applicable** |
| **600** | 31 | 33 | 35 | 33 | 32 | 36 | 33 | 32 | 31 | 34 | **100** | **33.0** | **4.9** | **-5.8** |
| **800** | 33 | 32 | 32 | 31 | 32 | 31 | 34 | 34 | 30 | 32 | **100** | **32.1** | **4.0** | **-2.9** |
| **1000** | 30 | 30 | 31 | 31 | 28 | 34 | 31 | 27 | 30 | 31 | **100** | **30.3** | **6.2** | **2.9** |
| **1200** | 17 | 19 | 16 | 20 | 17 | 19 | 17 | 18 | 18 | 18 | **100** | **17.9** | **6.7** | **42.6** |
| **1400** | 2 | 1 | 3 | 1 | 1 | 1 | 2 | 2 | 1 | 3 | **100** | **1.7** | **48.4** | **94.6** |

**Dunnett's MSD value:** 1.470

**PMSD:** 4.7

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 (10$^{th}$ percentile) = 13%.

Upper PMSD bound determined by USEPA (90$^{th}$ percentile) = 47%.

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

# Statistical Analyses

Entered and Reviewed by Jim Sumner

## Ceriodaphnia Survival and Reproduction Test-Reproduction

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 6/3/2025 | Test ID: | CdNaCICR | Sample ID: | REF-Ref Toxicant |
| End Date: | 6/10/2025 | 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 | 32.000 | 31.000 | 32.000 | 32.000 | 29.000 | 33.000 | 28.000 | 32.000 | 31.000 | 32.000 |
| 600 | 31.000 | 33.000 | 35.000 | 33.000 | 32.000 | 36.000 | 33.000 | 32.000 | 31.000 | 34.000 |
| 800 | 33.000 | 32.000 | 32.000 | 31.000 | 32.000 | 31.000 | 34.000 | 34.000 | 30.000 | 32.000 |
| 1000 | 30.000 | 30.000 | 31.000 | 31.000 | 28.000 | 34.000 | 31.000 | 27.000 | 30.000 | 31.000 |
| 1200 | 17.000 | 19.000 | 16.000 | 20.000 | 17.000 | 19.000 | 17.000 | 18.000 | 18.000 | 18.000 |
| 1400 | 2.000 | 1.000 | 3.000 | 1.000 | 1.000 | 1.000 | 2.000 | 2.000 | 1.000 | 3.000 |

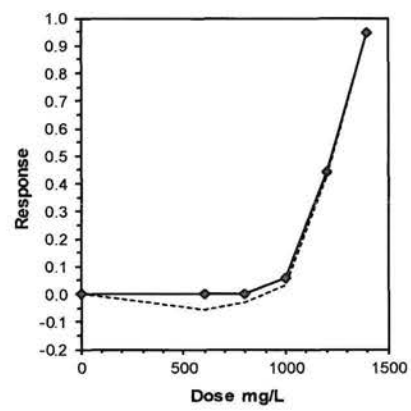
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| D-Control | 31.200 | 1.0000 | 31.200 | 28.000 | 33.000 | 4.965 | 10 | | | | 32.100 | 1.0000 |
| 600 | 33.000 | 1.0577 | 33.000 | 31.000 | 36.000 | 4.948 | 10 | -2.800 | 2.287 | 1.470 | 32.100 | 1.0000 |
| 800 | 32.100 | 1.0288 | 32.100 | 30.000 | 34.000 | 4.008 | 10 | -1.400 | 2.287 | 1.470 | 32.100 | 1.0000 |
| 1000 | 30.300 | 0.9712 | 30.300 | 27.000 | 34.000 | 6.233 | 10 | 1.400 | 2.287 | 1.470 | 30.300 | 0.9439 |
| *1200 | 17.900 | 0.5737 | 17.900 | 16.000 | 20.000 | 6.688 | 10 | 20.687 | 2.287 | 1.470 | 17.900 | 0.5576 |
| *1400 | 1.700 | 0.0545 | 1.700 | 1.000 | 3.000 | 48.428 | 10 | 45.885 | 2.287 | 1.470 | 1.700 | 0.0530 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates normal distribution (p > 0.01) | 0.63025 | 1.035 | -0.034 | 0.51873 |
| Bartlett's Test indicates equal variances (p = 0.26) | 6.49189 | 15.0863 | | |

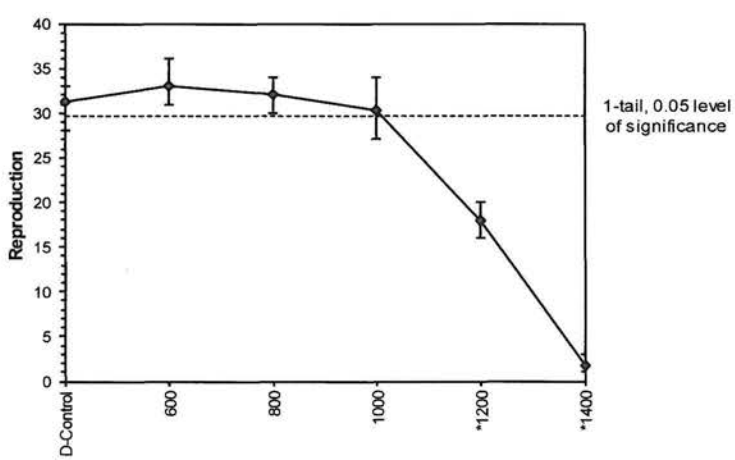
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 1000 | 1200 | 1095.45 | | 1.47012 | 0.04712 | 1543.67 | 2.06667 | 0.0E+00 | 5, 54 |
| Treatments vs D-Control | | | | | | | | | | |

### Linear Interpolation (200 Resamples)

| Point | mg/L | SD | 95% CL | | Skew |
|---|---|---|---|---|---|
| IC05 | 978.333 | 39.8132 | 895.994 | 1013.24 | -0.5323 |
| IC10 | 1022.74 | 9.10416 | 1006.21 | 1036.95 | -0.2776 |
| IC15 | 1048.63 | 7.97784 | 1033.08 | 1060.91 | -0.1532 |
| IC20 | 1074.52 | 7.13886 | 1059.98 | 1086.11 | -0.1834 |
| IC25 | 1100.4 | 6.45135 | 1086.88 | 1111.07 | -0.2077 |
| IC40 | 1178.06 | 5.7987 | 1165.57 | 1189.34 | 0.0503 |
| IC50 | 1222.84 | 4.15594 | 1214.22 | 1230.13 | -0.0782 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 312**

## *Daily Chemistry:*

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

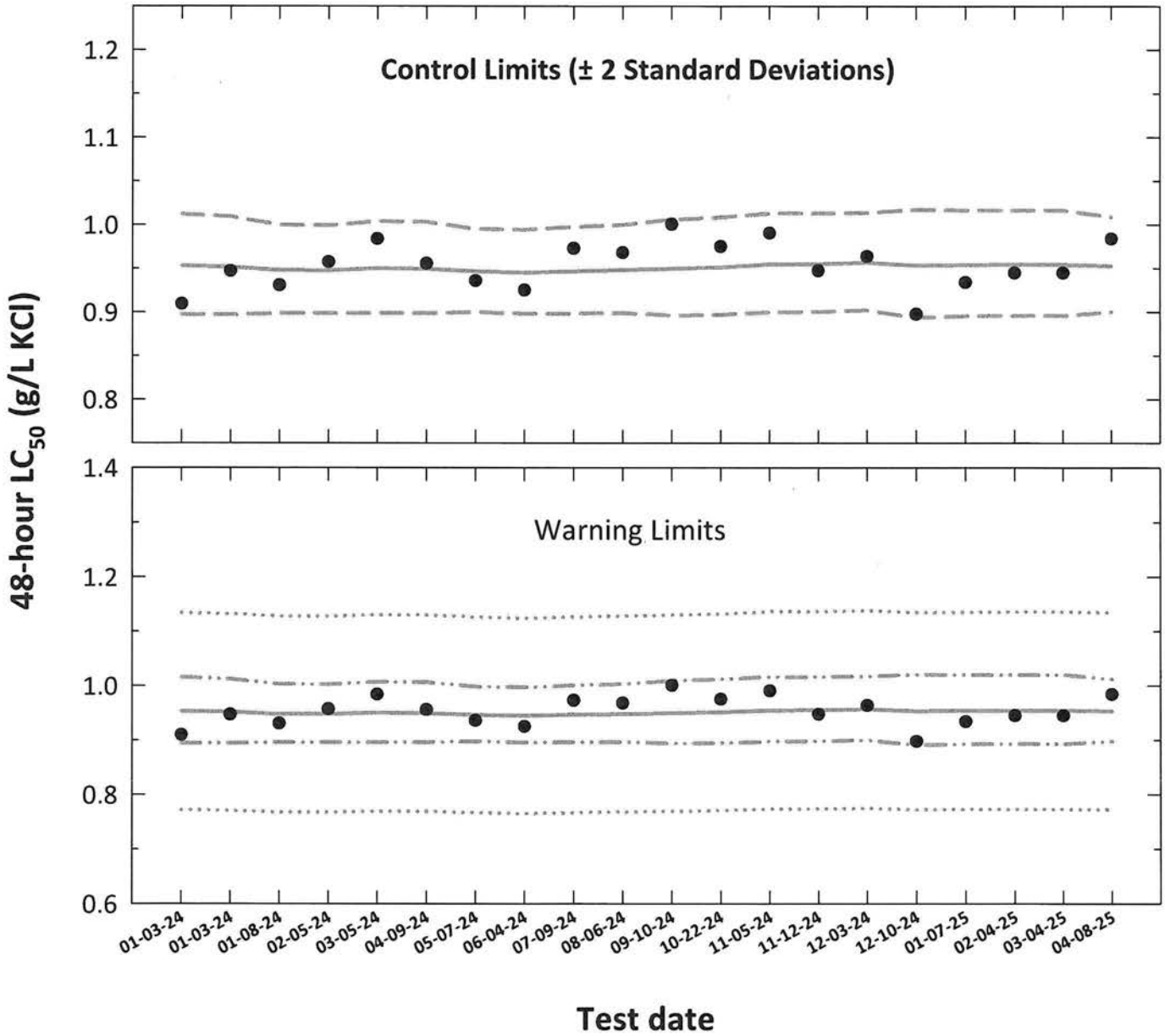
| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | XL | XL | XL | XL | XL | XL |
| Concentration | Parameter | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.74 | 7.85 | 7.75 | 7.87 | 7.79 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.6 | 8.1 | 8.4 | 8.1 | 8.4 | 8.1 |
| | Conductivity (µmhos/cm) | ~~1340~~ 294 (2006-05-25) | | 310 | | 313 | |
| | Alkalinity (mg $CaCO_3$/L) | 62 | | | | 59 | |
| | Hardness (mg $CaCO_3$/L) | 68 | | | (2006-05-25) | 90 | |
| | Temperature (°C) | 24.9 | 25.1 | 24.8 | 25.2 | 24.9 | 25.2 |
| 600 mg NaCl/L | pH (S.U.) | 7.84 | 7.84 | 7.82 | 7.87 | 7.85 | 7.84 |
| | Dissolved oxygen (mg/L) | 8.5 | 8.0 | 8.3 | 8.1 | 8.3 | 8.1 |
| | Conductivity (µmhos/cm) | 1340 | | 1400 | | 1400 | |
| | Temperature (°C) | 24.9 | 25.2 | 24.8 | 25.2 | 25.0 | 25.0 |
| 800 mg NaCl/L | pH (S.U.) | 7.87 | 7.82 | 7.84 | 7.88 | 7.86 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.5 | 8.0 | 8.3 | 8.1 | 8.3 | 8.1 |
| | Conductivity (µmhos/cm) | 1700 | | 1800 | | 1790 | |
| | Temperature (°C) | 25.0 | 25.2 | 24.9 | 25.0 | 24.9 | 24.8 |
| 1000 mg NaCl/L | pH (S.U.) | 7.87 | 7.83 | 7.86 | 7.88 | 7.89 | 7.88 |
| | Dissolved oxygen (mg/L) | 8.5 | 8.0 | 8.3 | 8.1 | 8.3 | 8.1 |
| | Conductivity (µmhos/cm) | 2040 | | 2150 | | 2160 | |
| | Temperature (°C) | 25.0 | 25.2 | 24.9 | 25.0 | 24.9 | 25.2 |
| 1200 mg NaCl/L | pH (S.U.) | 7.88 | 7.83 | 7.87 | 7.87 | 7.89 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.5 | 8.0 | 8.3 | 8.1 | 8.3 | 8.1 |
| | Conductivity (µmhos/cm) | 2390 | | 2490 | | 2510 | |
| | Temperature (°C) | 25.0 | 25.0 | 25.0 | 25.0 | 24.9 | 24.8 |
| 1400 mg NaCl/L | pH (S.U.) | 7.88 | 7.83 | 7.87 | 7.86 | 7.89 | 7.88 |
| | Dissolved oxygen (mg/L) | 8.5 | 8.0 | 8.3 | 8.1 | 8.3 | 8.0 |
| | Conductivity (µmhos/cm) | 2730 | | 2840 | | 2850 | |
| | Temperature (°C) | 25.0 | 24.9 | 25.0 | 24.9 | 24.9 | 25.1 |
| | | Initial | Final | Initial | Final | Initial | Final |

Environmental Testing Solutions, Inc.

**Species:** ***Ceriodaphnia dubia*** **CdNaClCR #: 312**

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | XL | BSL | BSL | BSL | BSL | XL | XL | XL |
| Concentration | Parameter | | | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.80 | 8.01 | 7.88 | 8.03 | 7.87 | 7.81 | 7.81 | 7.70 |
| | Dissolved oxygen (mg/L) | 8.4 | 8.0 | 8.3 | 8.0 | 8.4 | 7.9 | 8.3 | 7.9 |
| | Conductivity (µmhos/cm) | 298 | | 299 | | 295 | | 305 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 60 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | 06-10-25 XL | 90 | | | | | 06-10-25 XL |
| | Temperature (°C) | 24.8 | 24.9 | 24.7 | 25.0 | 24.9 | 24.9 | 24.8 | 25.1 |
| 600 mg NaCl/L | pH (S.U.) | 7.87 | 8.01 | 7.92 | 8.03 | 7.83 | 7.86 | 7.90 | 7.80 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.4 | 8.1 | 8.5 | 7.9 | 8.2 | 7.9 |
| | Conductivity (µmhos/cm) | 1350 | | 1370 | | 1350 | | 1380 | |
| | Temperature (°C) | 24.9 | 24.9 | 24.6 | 24.9 | 25.0 | 25.1 | 24.9 | 25.3 |
| 800 mg NaCl/L | pH (S.U.) | 7.89 | 8.01 | 7.97 | 8.02 | 7.99 | 7.87 | 7.93 | 7.80 |
| | Dissolved oxygen (mg/L) | 8.4 | 8.0 | 8.3 | 8.1 | 8.5 | 7.9 | 8.2 | 7.9 |
| | Conductivity (µmhos/cm) | 1710 | | 1710 | | 1710 | | 1780 | |
| | Temperature (°C) | 24.7 | 25.1 | 24.6 | 24.9 | 24.9 | 24.9 | 25.0 | 25.0 |
| 1000 mg NaCl/L | pH (S.U.) | 7.90 | 8.01 | 8.02 | 8.03 | 7.99 | 7.87 | 7.94 | 7.82 |
| | Dissolved oxygen (mg/L) | 8.4 | 8.0 | 8.3 | 8.1 | 8.5 | 7.9 | 8.2 | 7.9 |
| | Conductivity (µmhos/cm) | 2060 | | 2060 | | 2060 | | 2150 | |
| | Temperature (°C) | 24.7 | 24.8 | 24.6 | 25.2 | 24.9 | 25.0 | 24.9 | 25.0 |
| 1200 mg NaCl/L | pH (S.U.) | 7.90 | 8.01 | 8.02 | 8.03 | 7.99 | 7.88 | 7.93 | 7.83 |
| | Dissolved oxygen (mg/L) | 8.4 | 8.0 | 8.3 | 8.1 | 8.4 | 7.9 | 8.2 | 7.9 |
| | Conductivity (µmhos/cm) | 2420 | | 2440 | | 2420 | | 2520 | |
| | Temperature (°C) | 24.8 | 24.8 | 24.6 | 25.2 | 25.0 | 25.0 | 24.9 | 25.2 |
| 1400 mg NaCl/L | pH (S.U.) | 7.90 | 8.01 | 8.01 | 8.03 | 7.99 | 7.89 | 7.92 | 7.84 |
| | Dissolved oxygen (mg/L) | 8.4 | 8.1 | 8.3 | 8.1 | 8.4 | 7.9 | 8.2 | 7.9 |
| | Conductivity (µmhos/cm) | 2760 | | 2780 | | 2770 | | 2880 | |
| | Temperature (°C) | 24.8 | 24.8 | 24.8 | 25.0 | 25.0 | 25.1 | 25.0 | 25.2 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |

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




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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

| Test number | Test date | 48-hour $LC_{50}$ ToxCal Determination (g/L KCl) | $Log_{10}$ Conversion | | | Anti-logarithmic Values (g/L KCl) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 48-hour $LC_{50}$ | CT | S | CT | Control Limits | | Laboratory Calculated CV Warning Limits | | 75th Percentile CV Warning Limits | |
| | | | | | | | CT - 2S | CT + 2S | CT - 2CV | CT + 2CV | CT - $S_{A.75}$ | CT + $S_{A.75}$ |
| 1 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 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 |
| 10 | 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 |
| 11 | 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 |
| 12 | 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 |
| 13 | 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 |
| 14 | 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 |
| 15 | 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 |
| 16 | 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 |
| 17 | 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 |
| 18 | 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 |
| 19 | 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 |
| 20 | 04-08-25 | 0.9839 | -0.0070 | -0.0209 | 0.0124 | 0.9530 | 0.9003 | 1.0089 | 0.8977 | 1.0117 | 0.7720 | 1.1341 |

*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 # 177

### *Dilution Preparation:*

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

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

Stock solution INSS #: 2357

### *Chemical Analyses:*

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | EM | EM | EM |
| Control, MHSW | pH (S.U.) | 7.89 | 7.86 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 297 | | |
| | Alkalinity (mg/L $CaCO_3$) | 59 | | |
| | Hardness (mg/L $CaCO_3$) | 89 | | |
| | Temperature (°C) | 24.7 | 25.0 | 24.7 |
| 500 mg/L | pH (S.U.) | 8.02 | 7.78 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.4 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 1170 | | |
| | Temperature (°C) | 24.7 | 24.7 | 24.7 |
| 750 mg/L | pH (S.U.) | 8.01 | 7.79 | 7.83 |
| | Dissolved oxygen (mg/L) | 8.4 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 1560 | | |
| | Temperature (°C) | 24.9 | 24.7 | 24.7 |
| 1000 mg/L | pH (S.U.) | 8.01 | 7.76 | 7.82 |
| | Dissolved oxygen (mg/L) | 8.4 | 7.9 | 8.1 |
| | Conductivity (µmhos/cm) | 1970 | | |
| | Temperature (°C) | 24.8 | 24.9 | 24.9 |
| 1250 mg/L | pH (S.U.) | 8.01 | 7.78 | 7.84 |
| | Dissolved oxygen (mg/L) | 8.4 | 7.9 | 8.1 |
| | Conductivity (µmhos/cm) | 2440 | | |
| | Temperature (°C) | 24.8 | 24.9 | 24.6 |
| 1500 mg/L | pH (S.U.) | 8.02 | 7.87 | |
| | Dissolved oxygen (mg/L) | 8.4 | 7.9 | 04-09-26 |
| | Conductivity (µmhos/cm) | 2830 | | |
| | Temperature (°C) | 24.8 | 24.9 | |

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

### *Chemical analyses:*

| Parameter | Reporting limit | Method number | Meter | Serial numbe |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O G-2021 | YSI Model 52CE | 08A100271 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2021 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2021 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2021 | Not applicable | Not applicabl |
| Temperature | 0.1 °C | SM 2550B-2010 | Digital Thermometer | 13060468 |

ETS

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

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

PpKClAC # 171

| Hours | Date | Feeding | | Test Initiation or Termination | | Location Incubator/Shelf | Randomizing Template | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | | | |
| 0 Initiation | 04-08-25 | * 0500 | H | 0700 | H | 6E | Yellow | 04-02-25B |
| 24 | 04-09-25 | | | 0700 | H | | | |
| 48 Termination | 04-10-25 | | | 0655 | 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: | 03-26-25 |
| Age (1 to 14 days old): | 5 TO 6 DAYS |
| Hatch date and times: | 04-02-25 1205 TO 04-03-25 0822 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 8.19 |
| | 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 | 9 1d | 6 4d | 6 4d | 0 10d | 1 9d | 0 10d | 0 10d |
| 48 Termination | 10 | 10 | 10 | 10 | 10 | 9 | 6 | 5 1d | 0 | 0 1d | 0 | 0 |
| Mean Survival | 100% | | 100% | | 95% | | 55% | | 0% | | 0% | |

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

### *Statistics:*

| | |
|---|---|
| Method | PROBIT |
| Lower 95% confidence limit (mg KCl/L) | 915.6 |
| Upper 95% confidence limit (mg KCl/L) | 1046.9 |
| 48-hour $LC_{50}$ (mg KCl/L) | 983.9 |

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: | 4/8/2025 | Test ID: | PpKCIAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 4/10/2025 | 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 | 0.9000 |
| 1000 | 0.6000 | 0.6000 |
| 1250 | 0.0000 | 0.1000 |
| 1500 | 0.0000 | 0.0000 |

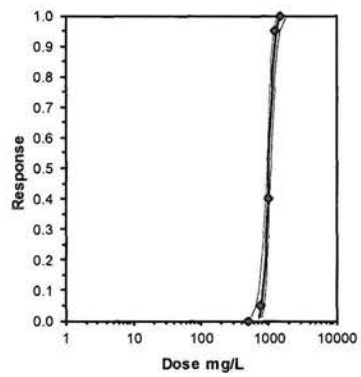
| | | | Transform: Arcsin Square Root | | | | | | 1-Tailed | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | | | | 0 | 20 |
| 500 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0.000 | 2.850 | 0.2077 | 0 | 20 |
| 750 | 0.9500 | 0.9500 | 1.3305 | 1.2490 | 1.4120 | 8.661 | 2 | 1.118 | 2.850 | 0.2077 | 1 | 20 |
| *1000 | 0.6000 | 0.6000 | 0.8861 | 0.8861 | 0.8861 | 0.000 | 2 | 7.216 | 2.850 | 0.2077 | 8 | 20 |
| *1250 | 0.0500 | 0.0500 | 0.2403 | 0.1588 | 0.3218 | 47.963 | 2 | 16.077 | 2.850 | 0.2077 | 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 | | | | |

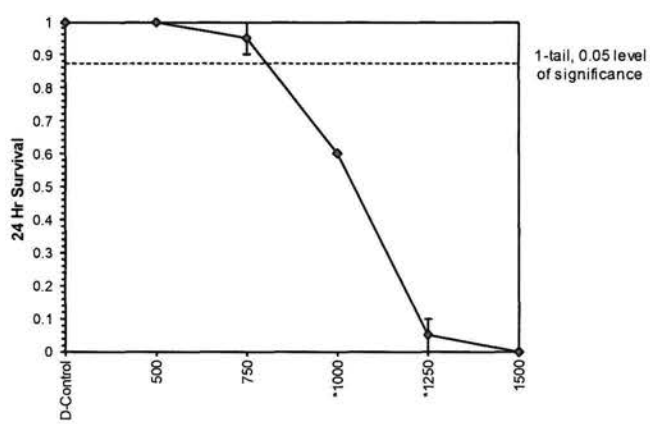
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 750 | 1000 | 866.025 | | 0.10341 | 0.10606 | 0.51158 | 0.00531 | 6.4E-05 | 4, 5 |
| Treatments vs D-Control | | | | | | | | | | |

| | | | Maximum Likelihood-Probit | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
| Slope | 15.2619 | 2.93363 | 9.51201 | 21.0118 | 0 | 1.29951 | 7.81472 | 0.72925 | 3.0034 | 0.06552 | 3 |
| Intercept | -40.838 | 8.84931 | -58.182 | -23.493 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 709.533 | 556.747 | 797.648 |
| EC05 | 3.355 | 786.368 | 653.728 | 863.28 |
| EC10 | 3.718 | 830.673 | 711.225 | 901.625 |
| EC15 | 3.964 | 861.968 | 752.193 | 929.262 |
| EC20 | 4.158 | 887.678 | 785.862 | 952.519 |
| EC25 | 4.326 | 910.346 | 815.389 | 973.599 |
| EC40 | 4.747 | 970.065 | 891.146 | 1033.03 |
| EC50 | 5.000 | 1007.86 | 936.295 | 1074.83 |
| EC60 | 5.253 | 1047.13 | 979.9 | 1122.7 |
| EC75 | 5.674 | 1115.82 | 1047.69 | 1217.67 |
| EC80 | 5.842 | 1144.32 | 1073.14 | 1260.76 |
| EC85 | 6.036 | 1178.45 | 1102.11 | 1314.67 |
| EC90 | 6.282 | 1222.84 | 1137.88 | 1387.97 |
| EC95 | 6.645 | 1291.74 | 1190.44 | 1507.48 |
| EC99 | 7.326 | 1431.62 | 1290.53 | 1767.14 |




## Dose-Response Plot



Environmental Testing Solutions, Inc.

# Statistical Analyses

Entered and Reviewed by Jim Sumner

## Acute Fathead Minnow Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 4/8/2025 | Test ID: | PpKCIAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 4/10/2025 | 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 | 0.9000 |
| 1000 | 0.6000 | 0.5000 |
| 1250 | 0.0000 | 0.0000 |
| 1500 | 0.0000 | 0.0000 |

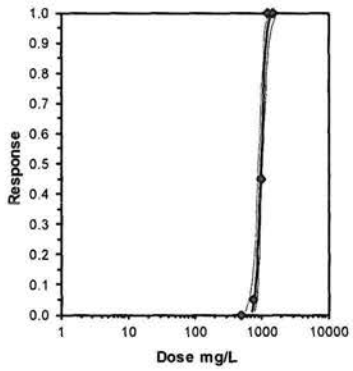
| | | | Transform: Arcsin Square Root | | | | | | 1-Tailed | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | | | | 0 | 20 |
| 500 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0.000 | 2.850 | 0.1726 | 0 | 20 |
| 750 | 0.9500 | 0.9500 | 1.3305 | 1.2490 | 1.4120 | 8.661 | 2 | 1.345 | 2.850 | 0.1726 | 1 | 20 |
| *1000 | 0.5500 | 0.5500 | 0.8357 | 0.7854 | 0.8861 | 8.518 | 2 | 9.513 | 2.850 | 0.1726 | 9 | 20 |
| *1250 | 0.0000 | 0.0000 | 0.1588 | 0.1588 | 0.1588 | 0.000 | 2 | 20.688 | 2.850 | 0.1726 | 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 | | | | |

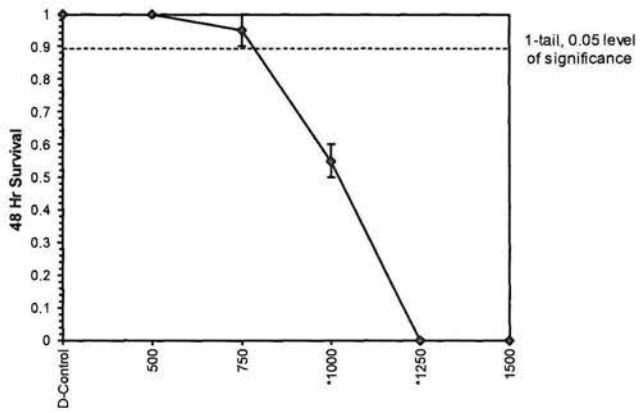
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 750 | 1000 | 866.025 | | 0.08088 | 0.08295 | 0.58948 | 0.00367 | 1.8E-05 | 4, 5 |
| Treatments vs D-Control | | | | | | | | | | |

| | | | Maximum Likelihood-Probit | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
| Slope | 17.1556 | 3.53793 | 10.2213 | 24.09 | 0 | 2.33295 | 7.81472 | 0.50624 | 2.99295 | 0.05829 | 5 |
| Intercept | -46.346 | 10.6305 | -67.182 | -25.51 | | | | | | | |
| TSCR | | | | | | | | | | | |

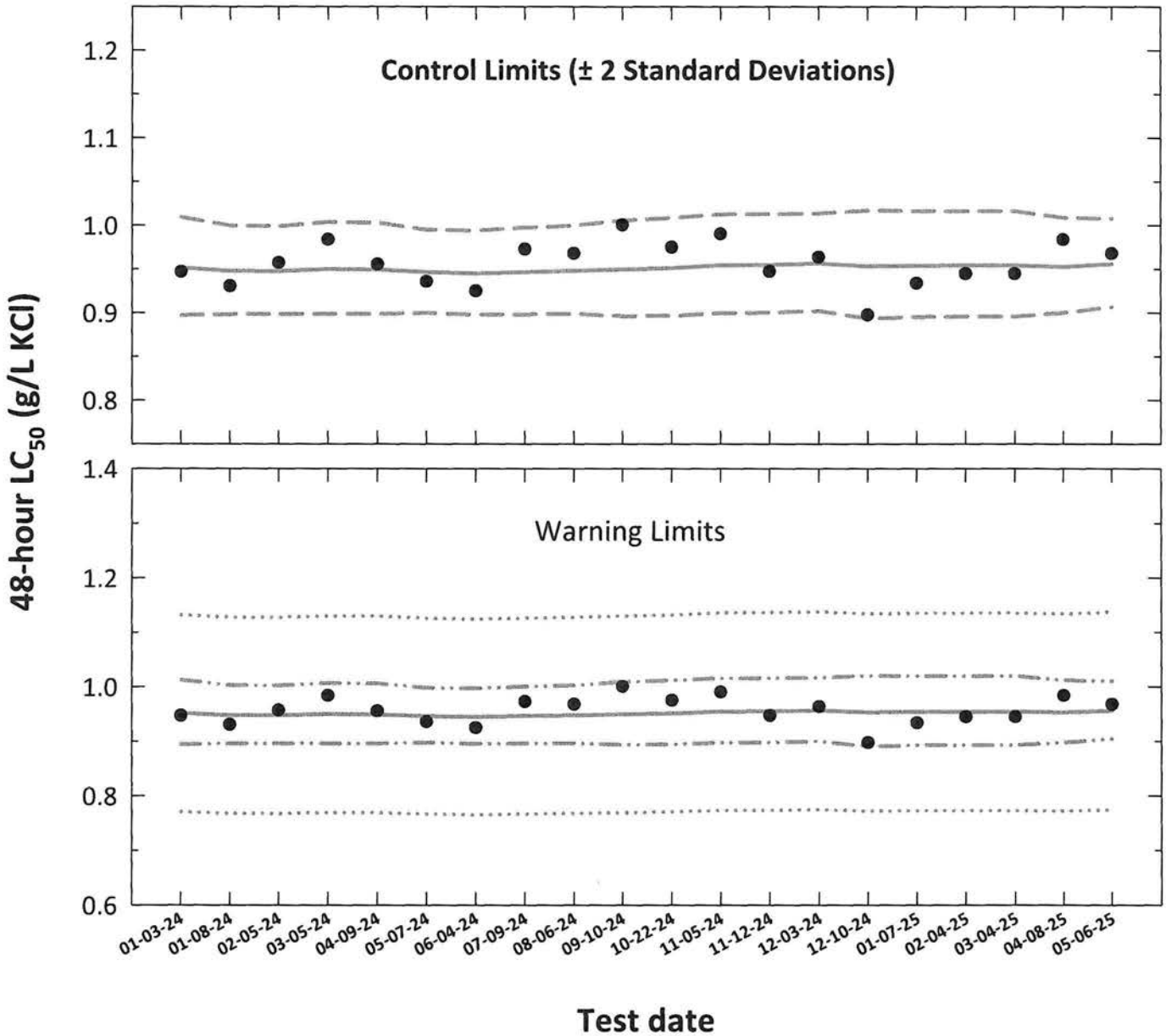
| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 720.03 | 565.312 | 803.757 |
| EC05 | 3.355 | 788.996 | 656.341 | 861.483 |
| EC10 | 3.718 | 828.422 | 709.823 | 895.059 |
| EC15 | 3.964 | 856.13 | 747.718 | 919.22 |
| EC20 | 4.158 | 878.811 | 778.73 | 939.544 |
| EC25 | 4.326 | 898.747 | 805.824 | 957.972 |
| EC40 | 4.747 | 951.011 | 874.854 | 1010.05 |
| EC50 | 5.000 | 983.905 | 915.584 | 1046.85 |
| EC60 | 5.253 | 1017.94 | 954.532 | 1089.16 |
| EC75 | 5.674 | 1077.13 | 1014.15 | 1173.46 |
| EC80 | 5.842 | 1101.57 | 1036.22 | 1211.73 |
| EC85 | 6.036 | 1130.75 | 1061.14 | 1259.59 |
| EC90 | 6.282 | 1168.57 | 1091.68 | 1324.53 |
| EC95 | 6.645 | 1226.96 | 1136.13 | 1430.06 |
| EC99 | 7.326 | 1344.48 | 1219.72 | 1657.63 |




### 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 | **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 |
| 2 | **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 |
| 3 | **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 |
| 4 | **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 |
| 5 | **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 |
| 6 | **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 |
| 7 | **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 |
| 8 | **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 |
| 9 | **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 |
| 10 | **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 |
| 11 | **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 |
| 12 | **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 |
| 13 | **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 |
| 14 | **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 |
| 15 | **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 |
| 16 | **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 |
| 17 | **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 |
| 18 | **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 |
| 19 | **04-08-25** | **0.9839** | -0.0070 | -0.0209 | 0.0124 | 0.9530 | 0.9003 | 1.0089 | 0.8977 | 1.0117 | 0.7720 | 1.1341 |
| 20 | **05-06-25** | **0.9680** | -0.0141 | -0.0195 | 0.0115 | 0.9560 | 0.9068 | 1.0079 | 0.9045 | 1.0103 | 0.7744 | 1.1377 |

*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

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# 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 #** 178

***Dilution Preparation:***

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

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

**Stock solution INSS #:** 2376

***Chemical Analyses:***

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | N | Em | Em |
| Control, MHSW | pH (S.U.) | 8.13 | 7.89 | 7.91 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 7.8 |
| | Conductivity (µmhos/cm) | 296 | | |
| | Alkalinity (mg/L $CaCO_3$) | 62 | | |
| | Hardness (mg/L $CaCO_3$) | 88 | | |
| | Temperature (°C) | 24.9 | 24.9 | 24.4 |
| 500 mg/L | pH (S.U.) | 8.10 | 7.86 | 7.88 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 7.9 |
| | Conductivity (µmhos/cm) | 1140 | | |
| | Temperature (°C) | 25.2 | 25.0 | 24.6 |
| 750 mg/L | pH (S.U.) | 8.10 | 7.87 | 7.85 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 7.8 |
| | Conductivity (µmhos/cm) | 1530 | | |
| | Temperature (°C) | 25.0 | 25.2 | 24.6 |
| 1000 mg/L | pH (S.U.) | 8.10 | 7.86 | 7.84 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 7.8 |
| | Conductivity (µmhos/cm) | 1970 | | |
| | Temperature (°C) | 25.0 | 24.8 | 24.3 |
| 1250 mg/L | pH (S.U.) | 8.10 | 7.84 | 7.87 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 7.8 |
| | Conductivity (µmhos/cm) | 2380 | | |
| | Temperature (°C) | 25.2 | 24.8 | 24.5 |
| 1500 mg/L | pH (S.U.) | 8.11 | 7.84 | |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | |
| | Conductivity (µmhos/cm) | 2800 | | |
| | Temperature (°C) | 25.0 | 24.8 | |

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

***Chemical analyses:***

| Parameter | Reporting limit | Method number | Meter | Serial numbe |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved oxygen | 1.0 mg/L | SM 4500-O G-2021 | YSI Model 52CE | 08A100271 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2021 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2021 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2021 | Not applicable | Not applicable |
| Temperature | 0.1°C | SM 2550B-2010 | Digital Thermometer | 13066468 |

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# 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 # 178

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 05-06-25 | * 0500 | H | 0700 | H | 1E | ANK | 04-30-25B |
| 24 | 06-07-25 | | | 0655 | H | | | |
| 48 Termination | 05-08-25 | | | 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: | 04-23-25 |
| Age (1 to 14 days old): | 6 TO 7 DAYS |
| Hatch date and times: | 04-25-25 1541 TO 04-30-25 0855 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 8.06 |
| | Temperature (°C): 24.2 |

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

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

| Hours | Control | | 500 mg/L | | 750 mg/L | | 1000 mg/L | | 1250 mg/L | | 1500 mg/L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | Replicate | | Replicate | | Replicate | | Replicate | | Replicate | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 24 | 10 | 10 | 10 | 10 | 10 | 9 1d | 6 4d | 5 5d | 0 10d | 1 9d | 0 10d | 0 10d |
| 48 Termination | 10 | 10 | 10 | 10 | 9 1d | 9 | 5 1d | 5 | 0 | 1 | 0 | 0 |
| Mean Survival | 100% | | 100% | | 90% | | 50% | | 5% | | 0% | |

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

### *Statistics:*

| | |
|---|---|
| Method | PROBIT |
| Lower 95% confidence limit (mg KCl/L) | 894.0 |
| Upper 95% confidence limit (mg KCl/L) | 1038.1 |
| 48-hour $LC_{50}$ (mg KCl/L) | 968.0 |

Comments:

Test Reviewed by: H

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# Statistical Analyses

Entered and Reviewed by Jim Sumner

## Acute Fathead Minnow Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/6/2025 | Test ID: | PpKCIAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/8/2025 | 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 | 0.9000 |
| 1000 | 0.6000 | 0.5000 |
| 1250 | 0.0000 | 0.1000 |
| 1500 | 0.0000 | 0.0000 |

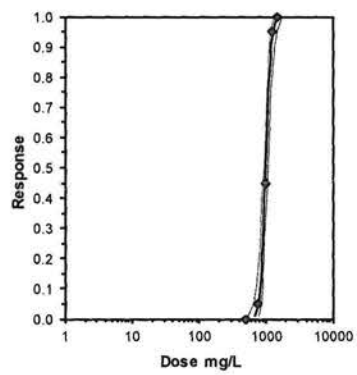
| | | | Transform: Arcsin Square Root | | | | | | 1-Tailed | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | | | | 0 | 20 |
| 500 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0.000 | 2.850 | 0.2267 | 0 | 20 |
| 750 | 0.9500 | 0.9500 | 1.3305 | 1.2490 | 1.4120 | 8.661 | 2 | 1.025 | 2.850 | 0.2267 | 1 | 20 |
| *1000 | 0.5500 | 0.5500 | 0.8357 | 0.7854 | 0.8861 | 8.518 | 2 | 7.246 | 2.850 | 0.2267 | 9 | 20 |
| *1250 | 0.0500 | 0.0500 | 0.2403 | 0.1588 | 0.3218 | 47.963 | 2 | 14.733 | 2.850 | 0.2267 | 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 | | | | |

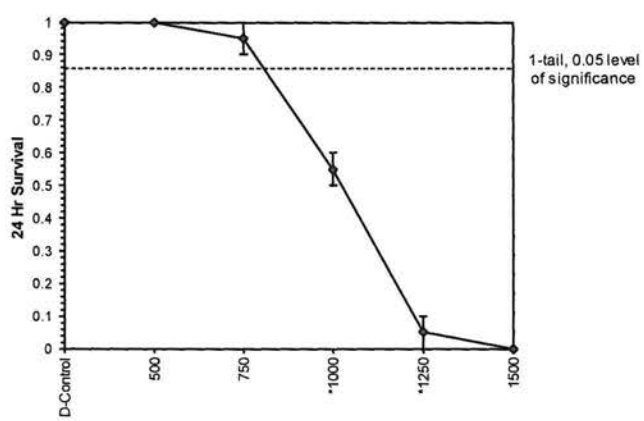
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 750 | 1000 | 866.025 | | 0.11636 | 0.11934 | 0.52116 | 0.00633 | 9.5E-05 | 4, 5 |
| Treatments vs D-Control | | | | | | | | | | |

| | | | | | Maximum Likelihood-Probit | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
| Slope | 15.1347 | 2.89305 | 9.4643 | 20.805 | 0 | 0.70045 | 7.81472 | 0.8731 | 2.99857 | 0.06607 | 3 |
| Intercept | -40.382 | 8.71294 | -57.46 | -23.305 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 699.621 | 549.012 | 786.967 |
| EC05 | 3.355 | 776.054 | 645.138 | 852.41 |
| EC10 | 3.718 | 820.156 | 702.155 | 890.672 |
| EC15 | 3.964 | 851.319 | 742.789 | 918.26 |
| EC20 | 4.158 | 876.928 | 776.188 | 941.482 |
| EC25 | 4.326 | 899.512 | 805.482 | 962.532 |
| EC40 | 4.747 | 959.033 | 880.665 | 1021.88 |
| EC50 | 5.000 | 996.72 | 925.504 | 1063.6 |
| EC60 | 5.253 | 1035.89 | 968.854 | 1111.34 |
| EC75 | 5.674 | 1104.43 | 1036.38 | 1205.94 |
| EC80 | 5.842 | 1132.87 | 1061.78 | 1248.83 |
| EC85 | 6.036 | 1166.95 | 1090.7 | 1302.51 |
| EC90 | 6.282 | 1211.29 | 1126.44 | 1375.48 |
| EC95 | 6.645 | 1280.13 | 1179 | 1494.51 |
| EC99 | 7.326 | 1419.98 | 1279.17 | 1753.28 |




### Dose-Response Plot



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# Statistical Analyses

Entered and Reviewed by Jim Sumner

## Acute Fathead Minnow Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/6/2025 | Test ID: | PpKCIAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/8/2025 | 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 | 0.5000 |
| 1250 | 0.0000 | 0.1000 |
| 1500 | 0.0000 | 0.0000 |

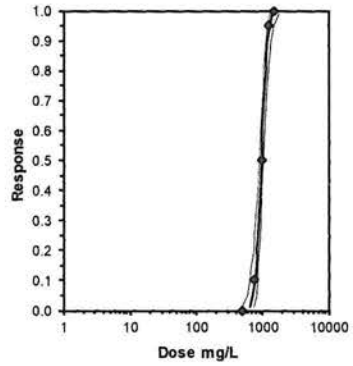
| | | | Transform: Arcsin Square Root | | | | | | 1-Tailed | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | | | | 0 | 20 |
| 500 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0.000 | 2.850 | 0.1469 | 0 | 20 |
| *750 | 0.9000 | 0.9000 | 1.2490 | 1.2490 | 1.2490 | 0.000 | 2 | 3.162 | 2.850 | 0.1469 | 2 | 20 |
| *1000 | 0.5000 | 0.5000 | 0.7854 | 0.7854 | 0.7854 | 0.000 | 2 | 12.159 | 2.850 | 0.1469 | 10 | 20 |
| *1250 | 0.0500 | 0.0500 | 0.2403 | 0.1588 | 0.3218 | 47.963 | 2 | 22.737 | 2.850 | 0.1469 | 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 | | | | |

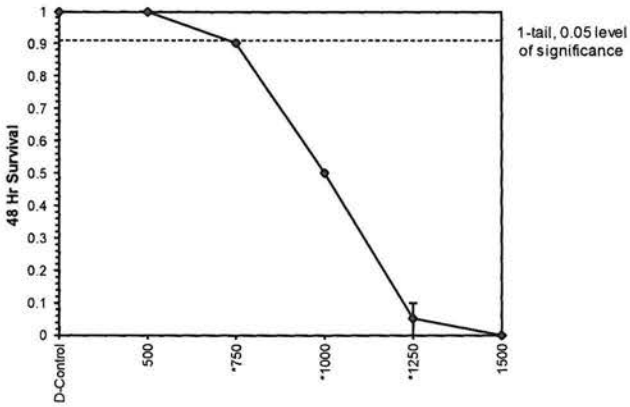
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 500 | 750 | 612.372 | | 0.06555 | 0.06723 | 0.51142 | 0.00266 | 1.2E-05 | 4, 5 |
| Treatments vs D-Control | | | | | | | | | | |

| | | | | | Maximum Likelihood-Probit | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
| Slope | 13.2148 | 2.40897 | 8.4932 | 17.9364 | 0 | 0.94914 | 7.81472 | 0.81355 | 2.98587 | 0.07567 | 3 |
| Intercept | -34.458 | 7.23006 | -48.629 | -20.287 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 645.405 | 498.603 | 734.866 |
| EC05 | 3.355 | 726.78 | 596.936 | 805.88 |
| EC10 | 3.718 | 774.274 | 656.126 | 847.698 |
| EC15 | 3.964 | 808.06 | 698.686 | 877.957 |
| EC20 | 4.158 | 835.96 | 733.899 | 903.478 |
| EC25 | 4.326 | 860.663 | 764.958 | 926.643 |
| EC40 | 4.747 | 926.194 | 845.444 | 992.045 |
| EC50 | 5.000 | 967.996 | 894.019 | 1038.06 |
| EC60 | 5.253 | 1011.68 | 941.438 | 1090.77 |
| EC75 | 5.674 | 1088.71 | 1016.28 | 1195.58 |
| EC80 | 5.842 | 1120.89 | 1044.74 | 1243.31 |
| EC85 | 6.036 | 1159.59 | 1077.35 | 1303.25 |
| EC90 | 6.282 | 1210.19 | 1117.94 | 1385.13 |
| EC95 | 6.645 | 1289.27 | 1178.14 | 1519.66 |
| EC99 | 7.326 | 1451.83 | 1294.34 | 1816.06 |




## Dose-Response Plot



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# Acute Reference Toxicant Control Chart
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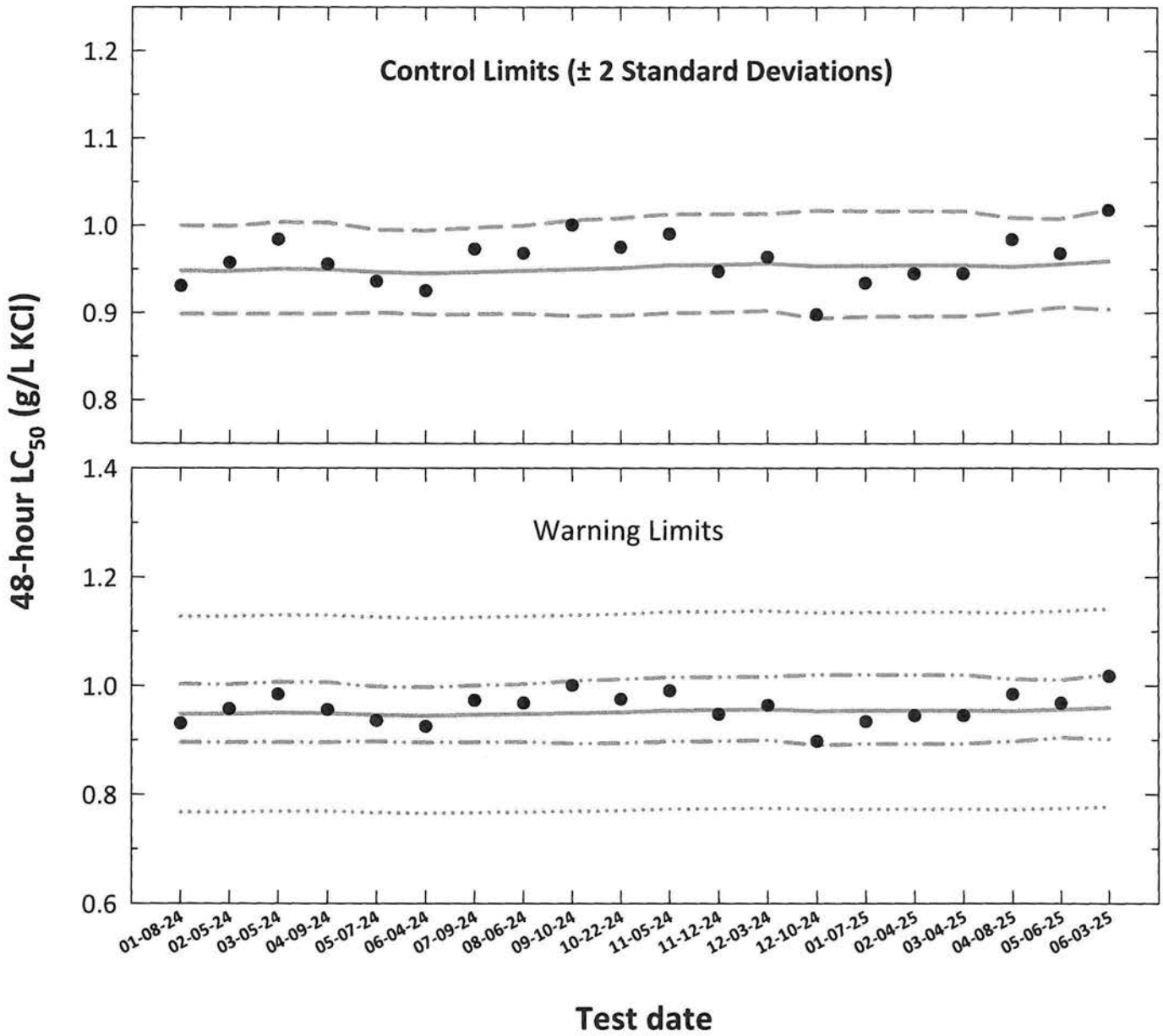


- ● **48-hour $LC_{50}$** = median lethal concentration. An estimation of the potassium chloride concentration which is lethal to 50% of the test organisms in 48-hours (calculated using ToxCalc).
- ——— **Central Tendency** (mean logarithmic $LC_{50}$ converted to anti-logarithmic values)
- — — — **Control 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)

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# *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 | **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 |
| 2 | **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 |
| 3 | **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 |
| 4 | **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 |
| 5 | **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 |
| 6 | **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 |
| 7 | **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 |
| 8 | **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 |
| 9 | **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 |
| 10 | **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 |
| 11 | **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 |
| 12 | **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 |
| 13 | **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 |
| 14 | **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 |
| 15 | **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 |
| 16 | **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 |
| 17 | **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 |
| 18 | **04-08-25** | **0.9839** | -0.0070 | -0.0209 | 0.0124 | 0.9530 | 0.9003 | 1.0089 | 0.8977 | 1.0117 | 0.7720 | 1.1341 |
| 19 | **05-06-25** | **0.9680** | -0.0141 | -0.0195 | 0.0115 | 0.9560 | 0.9068 | 1.0079 | 0.9045 | 1.0103 | 0.7744 | 1.1377 |
| 20 | **06-03-25** | **1.0177** | 0.0076 | -0.0180 | 0.0129 | 0.9595 | 0.9040 | 1.0183 | 0.9017 | 1.0208 | 0.7772 | 1.1418 |

*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 #** 179

### *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 #:** 2376

### *Chemical Analyses:*

| Concentration | | Hours 0 | 24 | 48 |
|---|---|---|---|---|
| | Analyst | XL | XL | XL |
| Control, MHSW | pH (S.U.) | 7.74 | 7.83 | 7.86 |
| | Dissolved oxygen (mg/L) | 8.6 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 244 | | |
| | Alkalinity (mg/L $CaCO_3$) | 62 | | |
| | Hardness (mg/L $CaCO_3$) | 88 | | |
| | Temperature (°C) | 24.7 | 24.9 | 25.0 |
| 500 mg/L | pH (S.U.) | 7.92 | 7.77 | 7.81 |
| | Dissolved oxygen (mg/L) | 8.6 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 1140 | | |
| | Temperature (°C) | 24.9 | 24.5 | 24.7 |
| 750 mg/L | pH (S.U.) | 7.91 | 7.77 | 7.81 |
| | Dissolved oxygen (mg/L) | 8.6 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 1540 | | |
| | Temperature (°C) | 24.9 | 24.7 | 24.7 |
| 1000 mg/L | pH (S.U.) | 7.90 | 7.78 | 7.81 |
| | Dissolved oxygen (mg/L) | 8.5 | 8.1 | 8.1 |
| | Conductivity (µmhos/cm) | 1920 | | |
| | Temperature (°C) | 24.6 | 24.9 | 24.9 |
| 1250 mg/L | pH (S.U.) | 7.90 | 7.76 | 7.80 |
| | Dissolved oxygen (mg/L) | 8.5 | 8.1 | 8.1 |
| | Conductivity (µmhos/cm) | 2350 | | |
| | Temperature (°C) | 24.8 | 24.8 | 24.6 |
| 1500 mg/L | pH (S.U.) | 7.90 | 7.74 | 7.80 |
| | Dissolved oxygen (mg/L) | 8.5 | 8.1 | 8.1 |
| | Conductivity (µmhos/cm) | 2750 | | |
| | Temperature (°C) | 24.6 | 24.8 | 24.6 |

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

### *Chemical analyses:*

| Parameter | Reporting limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-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 # 179

| Hours | Date | Feeding | | Test Initiation or Termination | | Location | Randomizing | MHSW Batch |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Incubator/Shelf | Template | |
| 0 Initiation | 06-03-25 | * 0505 | JI | 0705 | JI | 1C | ORANGE | 05-28-25A |
| 24 | 06-04-25 | | | 0707 | JI | | | |
| 48 Termination | 06-05-25 | | | 0706 | JI | | | |

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

### *Test Organism Information:*

| | |
|---|---|
| Organism Source: | In-house culture |
| Spawning date: | 05-20-25 |
| Age (1 to 14 days old): 6-7 | ~~7~~ 8 DAYS 06-03-25 JI |
| Hatch date and times: | 05-27-25 1140 TO 05-28-25 0908 |
| Average transfer volume: | < 0.25 mL |
| Transfer bowl information: | pH (S.U.): 8.04 |
| | Temperature (°C): 24.0 |

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

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

| Hours | Control | | 500 mg/L | | 750 mg/L | | 1000 mg/L | | 1250 mg/L | | 1500 mg/L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | | Replicate | | Replicate | | Replicate | | Replicate | | Replicate | |
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 Initiation | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 24 | 10 | 10 | 10 | 10 | 10 | 10 | 7 3d | 6 4d | 2 8d | 0 10d | 0 10d | 1 9d |
| 48 Termination | 10 | 10 | 10 | 10 | 10 | 10 | 5 2d | 4 2d | 2 | 0 | 0 | 1 |
| Mean Survival | 100% | | 100% | | 100% | | 45% | | 10% | | 5% | |

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

### *Statistics:*

| | |
|---|---|
| Method | PROBIT |
| Lower 95% confidence limit (mg KCl/L) | 941.1 |
| Upper 95% confidence limit (mg KCl/L) | 1090.4 |
| 48-hour $LC_{50}$ (mg KCl/L) | 1017.7 |

Comments:

Test Reviewed by: JI

Environmental Testing Solutions, Inc.

# Statistical Analyses

Entered and Reviewed by Jim Sumner

## Acute Fathead Minnow Test-24 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 6/3/2025 | Test ID: | PpKClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 6/5/2025 | 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 | 0.7000 | 0.6000 |
| 1250 | 0.2000 | 0.0000 |
| 1500 | 0.0000 | 0.1000 |

| | | | Transform: Arcsin Square Root | | | | | | 1-Tailed | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | | | | 0 | 20 |
| 500 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0.000 | 2.830 | 0.2952 | 0 | 20 |
| 750 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0.000 | 2.830 | 0.2952 | 0 | 20 |
| *1000 | 0.6500 | 0.6500 | 0.9386 | 0.8861 | 0.9912 | 7.916 | 2 | 4.539 | 2.830 | 0.2952 | 7 | 20 |
| *1250 | 0.1000 | 0.1000 | 0.3112 | 0.1588 | 0.4636 | 69.269 | 2 | 10.554 | 2.830 | 0.2952 | 18 | 20 |
| *1500 | 0.0500 | 0.0500 | 0.2403 | 0.1588 | 0.3218 | 47.963 | 2 | 11.234 | 2.830 | 0.2952 | 19 | 20 |

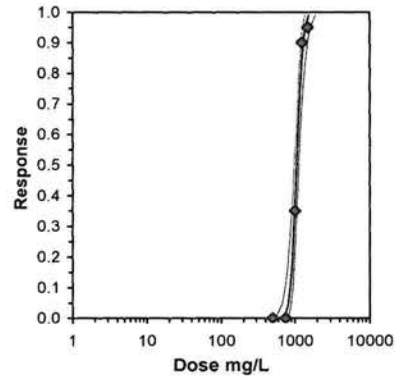
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Normality of the data set cannot be confirmed | | | | |
| Equality of variance cannot be confirmed | | | | |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 750 | 1000 | 866.025 | | 0.1673 | 0.17159 | 0.62087 | 0.01088 | 5.6E-05 | 5, 6 |
| Treatments vs D-Control | | | | | | | | | | |

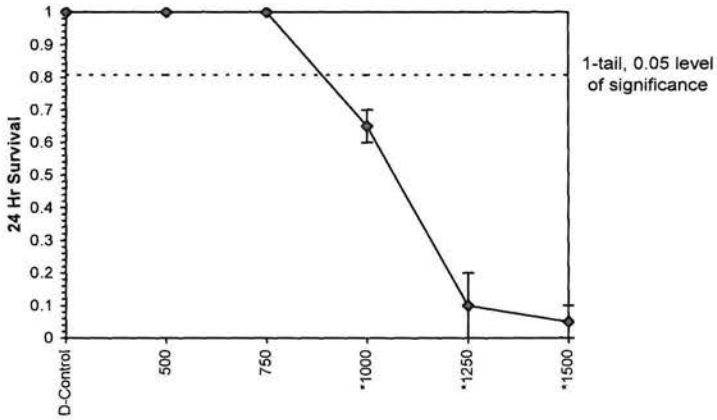
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 14.4223 | 2.71632 | 9.09835 | 19.7463 | 0 | 2.22616 | 7.81472 | 0.52681 | 3.02805 | 0.06934 | 4 |
| Intercept | -38.671 | 8.2605 | -54.862 | -22.481 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 735.775 | 574.963 | 830.097 |
| EC05 | 3.355 | 820.347 | 680.243 | 902.651 |
| EC10 | 3.718 | 869.336 | 743.06 | 945.125 |
| EC15 | 3.964 | 904.031 | 787.993 | 975.765 |
| EC20 | 4.158 | 932.591 | 825.023 | 1001.56 |
| EC25 | 4.326 | 957.81 | 857.572 | 1024.96 |
| EC40 | 4.747 | 1024.43 | 941.358 | 1091.04 |
| EC50 | 5.000 | 1066.71 | 991.422 | 1137.66 |
| EC60 | 5.253 | 1110.74 | 1039.82 | 1191.21 |
| EC75 | 5.674 | 1187.99 | 1115.17 | 1297.84 |
| EC80 | 5.842 | 1220.12 | 1143.54 | 1346.3 |
| EC85 | 6.036 | 1258.66 | 1175.92 | 1407 |
| EC90 | 6.282 | 1308.9 | 1216.05 | 1489.62 |
| EC95 | 6.645 | 1387.06 | 1275.29 | 1624.6 |
| EC99 | 7.326 | 1546.49 | 1388.88 | 1919.14 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

# Statistical Analyses

Entered and Reviewed by Jim Sumner

## Acute Fathead Minnow Test-48 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 6/3/2025 | Test ID: | PpKClAC | Sample ID: | REF-Ref Toxicant |
| End Date: | 6/5/2025 | 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 | 0.5000 | 0.4000 |
| 1250 | 0.2000 | 0.0000 |
| 1500 | 0.0000 | 0.1000 |

| | | | Transform: Arcsin Square Root | | | | | | 1-Tailed | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | | | | 0 | 20 |
| 500 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0.000 | 2.830 | 0.2941 | 0 | 20 |
| 750 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0.000 | 2.830 | 0.2941 | 0 | 20 |
| *1000 | 0.4500 | 0.4500 | 0.7351 | 0.6847 | 0.7854 | 9.685 | 2 | 6.513 | 2.830 | 0.2941 | 11 | 20 |
| *1250 | 0.1000 | 0.1000 | 0.3112 | 0.1588 | 0.4636 | 69.269 | 2 | 10.591 | 2.830 | 0.2941 | 18 | 20 |
| *1500 | 0.0500 | 0.0500 | 0.2403 | 0.1588 | 0.3218 | 47.963 | 2 | 11.273 | 2.830 | 0.2941 | 19 | 20 |

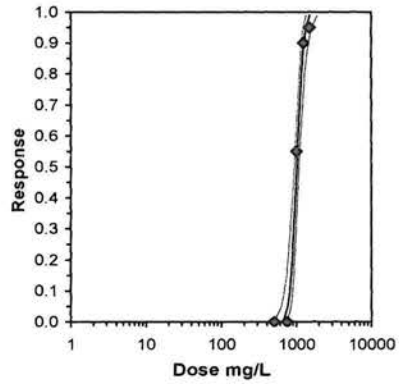
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Normality of the data set cannot be confirmed | | | | |
| Equality of variance cannot be confirmed | | | | |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 750 | 1000 | 866.025 | | 0.16649 | 0.17076 | 0.63724 | 0.0108 | 5.1E-05 | 5, 6 |
| Treatments vs D-Control | | | | | | | | | | |

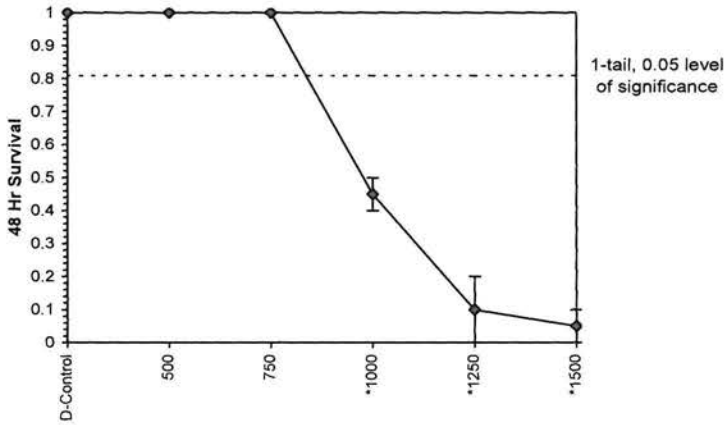
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 12.977 | 2.34084 | 8.38897 | 17.5651 | 0 | 3.42013 | 7.81472 | 0.33127 | 3.00762 | 0.07706 | 5 |
| Intercept | -34.03 | 7.07578 | -47.898 | -20.161 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 673.522 | 520.659 | 767.247 |
| EC05 | 3.355 | 760.094 | 624.836 | 842.865 |
| EC10 | 3.718 | 810.706 | 687.68 | 887.417 |
| EC15 | 3.964 | 846.743 | 732.928 | 919.659 |
| EC20 | 4.158 | 876.524 | 770.402 | 946.856 |
| EC25 | 4.326 | 902.907 | 803.481 | 971.546 |
| EC40 | 4.747 | 972.962 | 889.294 | 1041.29 |
| EC50 | 5.000 | 1017.7 | 941.114 | 1090.43 |
| EC60 | 5.253 | 1064.49 | 991.695 | 1146.8 |
| EC75 | 5.674 | 1147.08 | 1071.53 | 1259.04 |
| EC80 | 5.842 | 1181.61 | 1101.92 | 1310.18 |
| EC85 | 6.036 | 1223.17 | 1136.78 | 1374.41 |
| EC90 | 6.282 | 1277.54 | 1180.23 | 1462.18 |
| EC95 | 6.645 | 1362.6 | 1244.81 | 1606.41 |
| EC99 | 7.326 | 1537.75 | 1369.84 | 1924.53 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

**Surveillance and Corrective Action Report (SCAR)**

**SURVIELLANCE NUMBER:** SCAR-250618

**SURVIELLANCE:** Reference Toxicant Testing

**DATE OF INSPECTION:** 06-18-25

**TYPE OF INSPECTION:** Internal

**EVALUATORS:** Jim Sumner, Jaydon Perez

**LABORATORY PERSONNELL:** Jim Sumner, Jaydon Perez

## I. DEVIATION / DECREPANCY:

Errors in the *A. bahia* and *P. promelas* chronic PMSD calculation spreadsheets were identified.

- Calculations of upper and lower PMSD bounds were not correct.
- These spreadsheets are used to create PMSD control charts.

Error did not impact the validity of reference tests performed but identify a larger issue with how data is calculated in the laboratory.

## II. ROOT CAUSE INVESTIGATION:

Verbal instructions are provided for using spreadsheets used to validate results and calculate upper and lower control limits. These speadsheets have formulas for log transforming data used to calculate central tendencies/standard deviations and calculating control limits using anti-log values.

Spreadsheets are not locked to prevent an analyst from accidentally changing or deleting formulas. Individual spreadsheets do not have instructions for completing the data entry.

## III. EXECUTION:

Spreadsheets containing formulas are locked to prevent calculations from being altered.

Step by step instructions are included on the top of each spreadsheet tab.

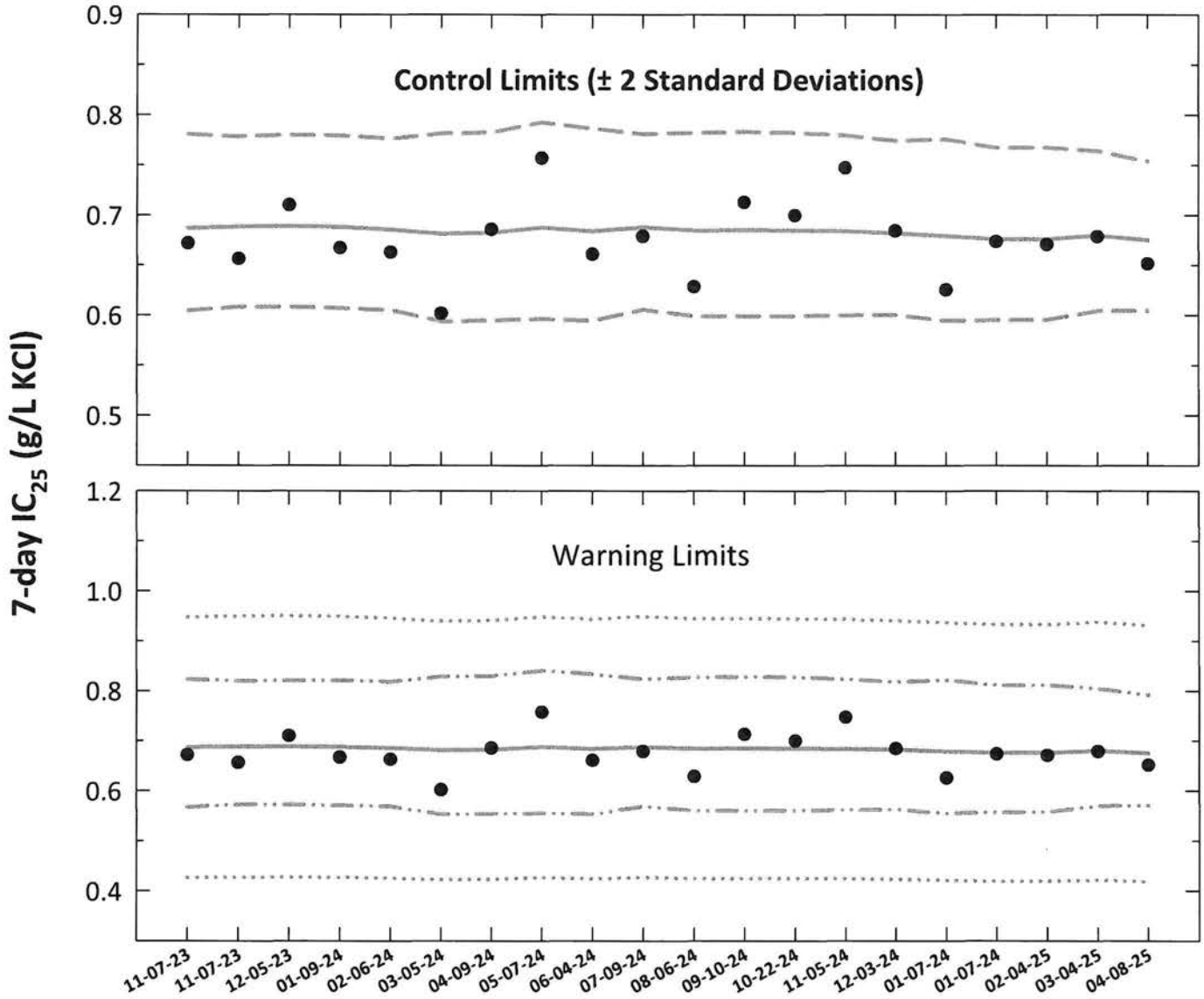
## IV. VERIFICATION:

Signature: _____ Laboratory Analyst  Date: 06-23-25

## V. CLOSURE:

Signature: _____ QA Officer  Date: 06-23-25

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




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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

| Test number | Test date | 7-day $IC_{25}$ ToxCal Determination (g/L KCl) | $Log_{10}$ Conversion | | | Anti-logarithmic Values (g/L KCl) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 7-day $IC_{25}$ | CT | S | CT | Control Limits | | Laboratory Calculated CV Warning Limits | | 75th Percentile CV Warning Limits | |
| | | | | | | | CT - 2S | CT + 2S | CT - 2CV | CT + 2CV | CT - $S_{A.75}$ | CT + $S_{A.75}$ |
| 1 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 06-04-24 | 0.6610 | -0.1798 | -0.1650 | 0.0303 | 0.6840 | 0.5949 | 0.7864 | 0.5537 | 0.8338 | 0.4241 | 0.9439 |
| 10 | 07-09-24 | 0.6789 | -0.1682 | -0.1624 | 0.0276 | 0.6880 | 0.6060 | 0.7811 | 0.5688 | 0.8233 | 0.4265 | 0.9494 |
| 11 | 08-06-24 | 0.6287 | -0.2015 | -0.1644 | 0.0289 | 0.6848 | 0.5994 | 0.7823 | 0.5602 | 0.8272 | 0.4246 | 0.9450 |
| 12 | 09-10-24 | 0.7128 | -0.1470 | -0.1642 | 0.0290 | 0.6852 | 0.5994 | 0.7832 | 0.5600 | 0.8283 | 0.4248 | 0.9456 |
| 13 | 10-22-24 | 0.6997 | -0.1551 | -0.1645 | 0.0289 | 0.6847 | 0.5994 | 0.7822 | 0.5601 | 0.8271 | 0.4245 | 0.9449 |
| 14 | 11-05-24 | 0.7473 | -0.1265 | -0.1648 | 0.0284 | 0.6842 | 0.6004 | 0.7797 | 0.5617 | 0.8238 | 0.4242 | 0.9442 |
| 15 | 12-03-24 | 0.6844 | -0.1647 | -0.1662 | 0.0276 | 0.6820 | 0.6006 | 0.7745 | 0.5626 | 0.8176 | 0.4228 | 0.9411 |
| 16 | 01-07-24 | 0.6255 | -0.2038 | -0.1680 | 0.0289 | 0.6792 | 0.5946 | 0.7757 | 0.5547 | 0.8213 | 0.4211 | 0.9372 |
| 17 | 01-07-24 | 0.6737 | -0.1715 | -0.1700 | 0.0276 | 0.6760 | 0.5955 | 0.7675 | 0.5569 | 0.8113 | 0.4191 | 0.9329 |
| 18 | 02-04-25 | 0.6706 | -0.1735 | -0.1699 | 0.0275 | 0.6762 | 0.5956 | 0.7676 | 0.5571 | 0.8114 | 0.4192 | 0.9331 |
| 19 | 03-04-25 | 0.6786 | -0.1684 | -0.1676 | 0.0254 | 0.6798 | 0.6047 | 0.7643 | 0.5693 | 0.8041 | 0.4215 | 0.9381 |
| 20 | 04-08-25 | 0.6516 | -0.1860 | -0.1705 | 0.0239 | 0.6753 | 0.6048 | 0.7540 | 0.5708 | 0.7919 | 0.4187 | 0.9319 |

*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.

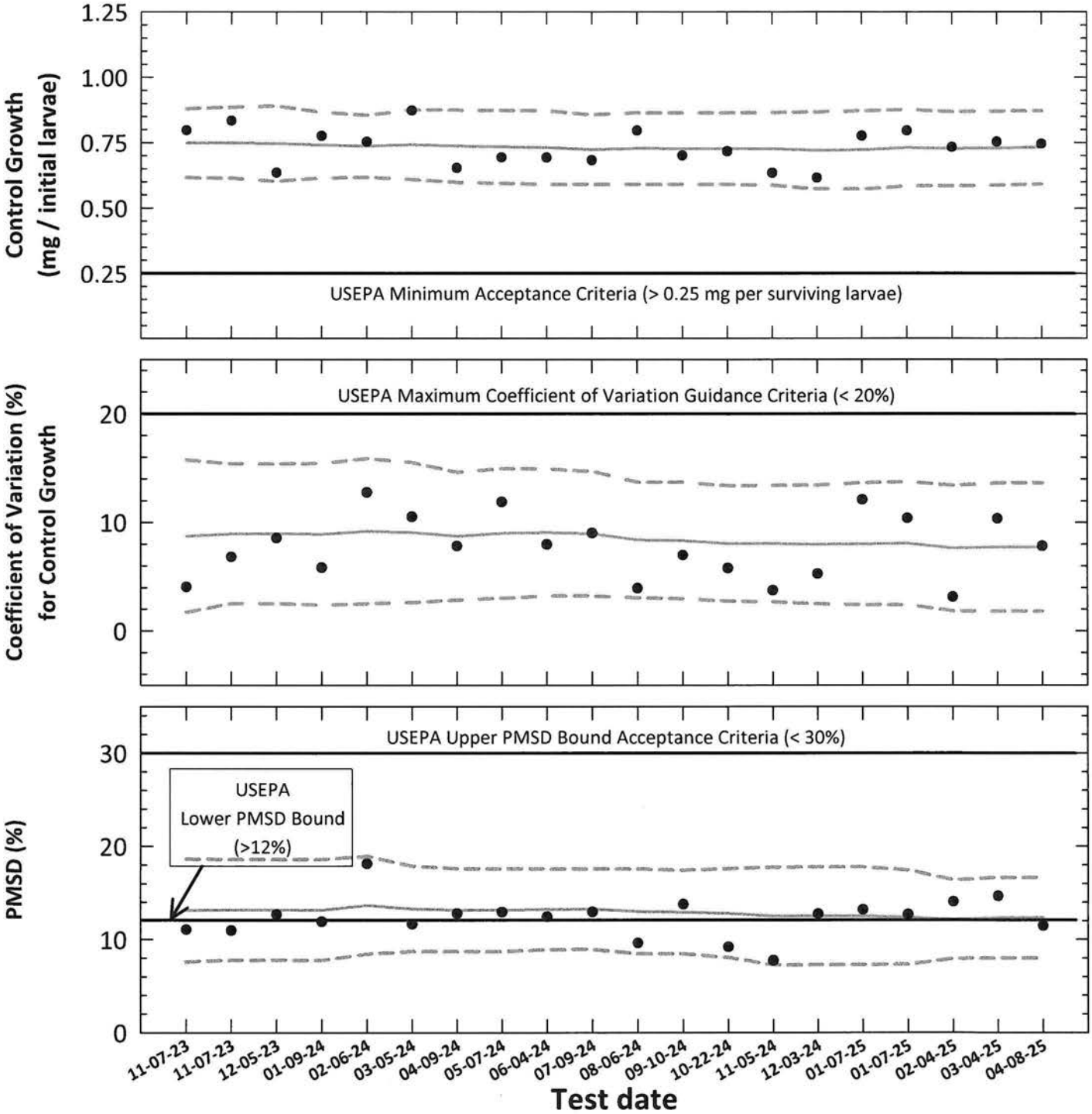
**$S_{A.75}$** = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

**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 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 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 |
| 9 | 06-04-24 | 100 | 0.693 | 8.0 | 0.0862 | 12.4 | 0.732 | 0.591 | 0.872 | 9.1 | 3.2 | 14.9 | 13.2 | 8.9 | 17.5 |
| 10 | 07-09-24 | 100 | 0.683 | 9.0 | 0.0883 | 12.9 | 0.724 | 0.591 | 0.857 | 9.0 | 3.2 | 14.7 | 13.2 | 8.9 | 17.5 |
| 11 | 08-06-24 | 100 | 0.796 | 3.9 | 0.0766 | 9.6 | 0.728 | 0.591 | 0.865 | 8.4 | 3.0 | 13.7 | 13.0 | 8.4 | 17.5 |
| 12 | 09-10-24 | 100 | 0.701 | 7.0 | 0.0964 | 13.8 | 0.728 | 0.591 | 0.865 | 8.3 | 2.9 | 13.7 | 12.9 | 8.5 | 17.4 |
| 13 | 10-22-24 | 100 | 0.717 | 5.8 | 0.0659 | 9.2 | 0.727 | 0.590 | 0.864 | 8.1 | 2.7 | 13.4 | 12.8 | 8.0 | 17.6 |
| 14 | 11-05-24 | 100 | 0.635 | 3.7 | 0.0491 | 7.7 | 0.727 | 0.588 | 0.866 | 8.0 | 2.7 | 13.4 | 12.5 | 7.3 | 17.8 |
| 15 | 12-03-24 | 100 | 0.615 | 5.3 | 0.0785 | 12.8 | 0.721 | 0.573 | 0.868 | 8.0 | 2.5 | 13.5 | 12.5 | 7.3 | 17.8 |
| 16 | 01-07-25 | 100 | 0.776 | 12.1 | 0.1024 | 13.2 | 0.723 | 0.573 | 0.872 | 8.0 | 2.4 | 13.7 | 12.5 | 7.3 | 17.8 |
| 17 | 01-07-25 | 100 | 0.796 | 10.4 | 0.1010 | 12.7 | 0.731 | 0.585 | 0.877 | 8.1 | 2.4 | 13.8 | 12.4 | 7.3 | 17.4 |
| 18 | 02-04-25 | 100 | 0.733 | 3.1 | 0.1030 | 14.1 | 0.727 | 0.585 | 0.869 | 7.6 | 1.8 | 13.4 | 12.2 | 7.9 | 16.4 |
| 19 | 03-04-25 | 100 | 0.754 | 10.4 | 0.1103 | 14.6 | 0.729 | 0.588 | 0.871 | 7.7 | 1.8 | 13.6 | 12.3 | 8.0 | 16.7 |
| 20 | 04-08-25 | 100 | 0.746 | 7.8 | 0.0855 | 11.5 | 0.733 | 0.593 | 0.873 | 7.7 | 1.8 | 13.7 | 12.3 | 8.0 | 16.7 |

*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 Surnior

Environmental Testing Solutions, Inc.

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

## Species: *Pimephales promelas*

PpKClCR Test Number: **127**

| ***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: | 03-31-25 | *Artemia* CHM number: | CHM1385 |
| Hatch dates and times: | 04-07-25 1440 TO 04-08-25 0455 | ***Drying information for weight determination:*** | |
| Transfer vessel information: | pH = 8.19 S.U.<br>Temperature = 24.0 °C | Date / Time in oven: | 04-15-25 0616 |
| | | *Initial oven temperature: | 60 °C |
| Average transfer volume: | < 0.25 mL | Date / Time out of oven: | 04-16-25 0620 |
| | | *Final oven temperature: | 60 °C |
| | | Total drying time: | 24-HOURS |

*60°C Oven, Thermometer SN: 14-985B5

***Daily feeding and renewal information:***

| Day | Date | Morning feeding | | Afternoon feeding | | Test initiation, renewal, or termination | | MHSW batch used |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Time | Analyst | |
| 0 | 04-08-25 | 0500 | H | 1530 | H | 0715 | H | 04-02-25 B |
| 1 | 04-09-25 | 0500 | H | 1510 | H | 0715 | H | ↓ |
| 2 | 04-10-25 | 0500 | H | 1400 | H | 0710 | H | 04-02-25 D |
| 3 | 04-11-25 | 0500 | H | 1215 | H | 0700 | H | ↓ |
| 4 | 04-12-25 | 0530 | H | 1230 | H | 0730 | H | 04-10-25 A |
| 5 | 04-13-25 | 0500 | H | 1100 | H | 0700 | H | ↓ |
| 6 | 04-14-25 | 0500 | H | 1100 | H | 0700 | H | ↓ |
| 7 | 04-15-25 | | | | | 0530 | H | |

***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) | ~~768.0~~ 764.0 |
| Average weight per initial larvae: | 0.746 | | **NOEC** (mg/L KCl) | 600 |
| Average weight per surviving larvae: | 0.746 | ≥ 0.25 mg/larvae | **LOEC** (mg/L KCl) | 750 |
| | | | **ChV** (mg/L KCl) | 670.8 |
| | | | **$IC_{25}$** (mg/L KCl) | 651.6 |

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **127**

***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)<br>Tray color code: Light pink<br>Analyst: XL<br>Date: 03-20-25 | 14.92 | 13.67 | 11.45 | 13.12 | 13.49 | 13.96 | 15.22 | 11.78 | 13.67 | 12.62 | 13.75 | 13.44 |
| *B = Pan + Larvae weight (mg)<br>Analyst: XL<br>Date: 04-17-25 | 21.78 | 20.80 | 19.10 | 21.30 | 23.07 | 22.15 | 24.04 | 21.46 | 22.06 | 20.89 | 22.09 | 21.89 |
| C = Larvae weight (mg) = B – A<br>Analyst: JT | 6.86 | 7.13 | 7.65 | 8.18 | 9.58 | 8.19 | 8.82 | 9.68 | 8.39 | 8.27 | 8.34 | 8.45 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JT | 0.686 | 0.713 | 0.765 | 0.818 | 0.958 | 0.819 | 0.882 | 0.968 | 0.839 | 0.827 | 0.834 | 0.845 |
| Average weight per initial number of larvae (mg) / Percent reduction from control (%) | 0.746 | | | | 0.907 | | -21.62 | | 0.836 | | -12.27 | |

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

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

***Comments:***

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **127**

***Survival and Growth Data***

| Day | 600 mg KCl/L | | | | 750 mg KCl/L | | | | 900 mg KCl/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 9 1d | 9 1d | 9 1d | 9 1d | 4 6d | 4 6d | 4 6d | 4 6d |
| 2 | 10 | 10 | 10 | 10 | 8 1d | 8 1d | 9 | 8 1d | 3 1d | 3 1d | 3 1d | 3 1d |
| 3 | 10 | 10 | 10 | 10 | 8 | 8 | 7 2d | 8 | 3 | 3 | 3 | 3 |
| 4 | 10 | 10 | 10 | 10 | 8 | 7 1d | 7 | 7 1d | 2 1d | 3 | 3 | 3 |
| 5 | 10 | 10 | 10 | 10 | 7 1d | 7 | 7 | 6 1d | 1 1d | 1 2d | 2 1d | 2 1d |
| 6 | 10 | 10 | 10 | 9 1d | 7 | 7 | 7 | 6 | 1 | 1 | 2 | 2 |
| 7 | 9 1d | 10 | 10 | 9 | 6 1d | 6 1d | 6 1d | 5 1d | 1 | 1 | 2 | 1 1d |
| *A = Pan weight (mg)<br>Tray color code: light pink<br>Analyst: XL<br>Date: 03-20-25 | 13.50 | 13.29 | 13.35 | 15.65 | 12.82 | 14.32 | 14.31 | 14.93 | 11.29 | 13.45 | 15.38 | 13.09 |
| *B = Pan + Larvae weight (mg)<br>Analyst: XL<br>Date: 04-17-25 | 20.34 | 21.30 | 20.56 | 22.60 | 17.14 | 18.15 | 19.28 | 18.84 | 12.32 | 14.39 | 17.24 | 13.78 |
| C = Larvae weight (mg) = B – A<br>Analyst: JL | 6.84 | 8.01 | 7.21 | 6.95 | 4.32 | 3.83 | 4.97 | 3.91 | 1.03 | 0.94 | 1.86 | 0.69 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: A | 0.684 | 0.801 | 0.721 | 0.695 | 0.432 | 0.383 | 0.497 | 0.391 | 0.103 | 0.094 | 0.186 | 0.069 |
| Average weight per initial number of larvae (mg) | 0.725 | | | | 0.426 | | | | 0.113 | | | |
| Percent reduction from control (%) | | | 2.77. | | | | 42.97. | | | | 84.87. | |

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

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

***Comments:***

Environmental Testing Solutions, Inc.

**Species: *Pimephales promelas*** PpKClCR Test Number: **127**

***Survival and Growth Data***

| Day | 1050 mg KCl/L | | | |
|---|---|---|---|---|
| | Y | Z | AA | BB |
| 0 | 10 | 10 | 10 | 10 |
| 1 | 2 sd | 3 rd | 2 sd | 2 sd |
| 2 | 1 d | 2 d | 2 | 1 d |
| 3 | 1 | 1 d | 1 d | 1 |
| 4 | 1 | 0 d | 1 | 1 |
| 5 | 0 kd | 0 | 1 | 1 |
| 6 | 0 | 0 | 1 | 1 |
| 7 | 0 | 0 | 0 d | 0 d |
| *A = Pan weight (mg)<br>Tray color code: light pink<br>Analyst: XL<br>Date: 03-20-25 | 13.33 | 12.72 | 13.34 | 12.74 |
| *B = Pan + Larvae weight (mg)<br>Analyst:<br>Date: | | | | |
| C = Larvae weight (mg) = B – A<br>Analyst: | | | | 04-15-25 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: | 0 | 0 | 0 | 0 |
| Average weight per initial number of larvae (mg) / Percent reduction from control (%) | 0 | | 100% | |

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

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

| ***Comments:*** |
|---|
| |
| |
| |

Environmental Testing Solutions, Inc.

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

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

Test number: 127

Test dates: April 08-15, 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 | 14.92 | 21.78 | 6.86 | 0.686 | 0.746 | 7.8 | 0.686 | 100.0 | 0.746 | 7.8 | Not applicable |
| | B | 10 | 10 | 13.67 | 20.80 | 7.13 | 0.713 | | | 0.713 | | | | |
| | C | 10 | 10 | 11.45 | 19.10 | 7.65 | 0.765 | | | 0.765 | | | | |
| | D | 10 | 10 | 13.12 | 21.30 | 8.18 | 0.818 | | | 0.818 | | | | |
| 300 | E | 10 | 10 | 13.49 | 23.07 | 9.58 | 0.958 | 0.907 | 7.7 | 0.958 | 100.0 | 0.907 | 7.7 | -21.6 |
| | F | 10 | 10 | 13.96 | 22.15 | 8.19 | 0.819 | | | 0.819 | | | | |
| | G | 10 | 10 | 15.22 | 24.04 | 8.82 | 0.882 | | | 0.882 | | | | |
| | H | 10 | 10 | 11.78 | 21.46 | 9.68 | 0.968 | | | 0.968 | | | | |
| 450 | I | 10 | 10 | 13.67 | 22.06 | 8.39 | 0.839 | 0.836 | 0.9 | 0.839 | 100.0 | 0.836 | 0.9 | -12.2 |
| | J | 10 | 10 | 12.62 | 20.89 | 8.27 | 0.827 | | | 0.827 | | | | |
| | K | 10 | 10 | 13.75 | 22.09 | 8.34 | 0.834 | | | 0.834 | | | | |
| | L | 10 | 10 | 13.44 | 21.89 | 8.45 | 0.845 | | | 0.845 | | | | |
| 600 | M | 10 | 9 | 13.50 | 20.34 | 6.84 | 0.760 | 0.764 | 4.3 | 0.684 | 95.0 | 0.725 | 7.3 | 2.7 |
| | N | 10 | 10 | 13.29 | 21.30 | 8.01 | 0.801 | | | 0.801 | | | | |
| | O | 10 | 10 | 13.35 | 20.56 | 7.21 | 0.721 | | | 0.721 | | | | |
| | P | 10 | 9 | 15.65 | 22.60 | 6.95 | 0.772 | | | 0.695 | | | | |
| 750 | Q | 10 | 6 | 12.82 | 17.14 | 4.32 | 0.720 | 0.742 | 11.1 | 0.432 | 57.5 | 0.426 | 12.2 | 42.9 |
| | R | 10 | 6 | 14.32 | 18.15 | 3.83 | 0.638 | | | 0.383 | | | | |
| | S | 10 | 6 | 14.31 | 19.28 | 4.97 | 0.828 | | | 0.497 | | | | |
| | T | 10 | 5 | 14.93 | 18.84 | 3.91 | 0.782 | | | 0.391 | | | | |
| 900 | U | 10 | 1 | 11.29 | 12.32 | 1.03 | 1.030 | 0.898 | 16.2 | 0.103 | 12.5 | 0.113 | 44.9 | 84.8 |
| | V | 10 | 1 | 13.45 | 14.39 | 0.94 | 0.940 | | | 0.094 | | | | |
| | W | 10 | 2 | 15.38 | 17.24 | 1.86 | 0.930 | | | 0.186 | | | | |
| | X | 10 | 1 | 13.09 | 13.78 | 0.69 | 0.690 | | | 0.069 | | | | |
| 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:** 0.0855 MSD = Minimum Significant Difference

**PMSD:** 11.5 PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Josh Summer

ETS Environmental Testing Solutions, Inc.

# Statistical Analyses

Entered and Reviewed by Jim Sumner

## Larval Fish Growth and Survival Test-7 Day Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 4/8/2025 | Test ID: | PpKCICR | Sample ID: | REF-Ref Toxicant |
| End Date: | 4/15/2025 | 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 | 1.0000 | 1.0000 | 0.9000 |
| 750 | 0.6000 | 0.6000 | 0.6000 | 0.5000 |
| 900 | 0.1000 | 0.1000 | 0.2000 | 0.1000 |
| 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 | 0.5750 | 0.5750 | 0.8609 | 0.7854 | 0.8861 | 5.847 | 4 | 10.00 | 10.00 | 17 | 40 |
| *900 | 0.1250 | 0.1250 | 0.3572 | 0.3218 | 0.4636 | 19.861 | 4 | 10.00 | 10.00 | 35 | 40 |
| 1050 | 0.0000 | 0.0000 | 0.1588 | 0.1588 | 0.1588 | 0.000 | 4 | | | 40 | 40 |

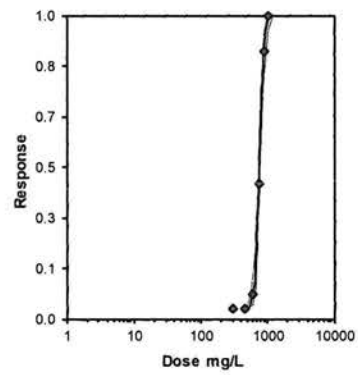
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.87948 | 0.884 | 0.32954 | 0.7878 |
| Equality of variance cannot be confirmed | | | | |

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

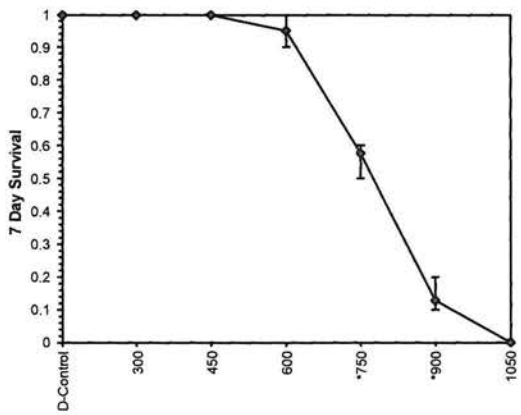
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 16.8491 | 2.16954 | 12.5968 | 21.1014 | 0 | 0.6573 | 9.48773 | 0.95649 | 2.88308 | 0.05935 | 3 |
| Intercept | -43.577 | 6.27577 | -55.878 | -31.277 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 555.921 | 491.988 | 599.902 |
| EC05 | 3.355 | 610.183 | 555.781 | 647.929 |
| EC10 | 3.718 | 641.242 | 592.635 | 675.619 |
| EC15 | 3.964 | 663.086 | 618.547 | 695.331 |
| EC20 | 4.158 | 680.977 | 639.669 | 711.716 |
| EC25 | 4.326 | 696.709 | 658.096 | 726.375 |
| EC40 | 4.747 | 737.983 | 705.228 | 766.498 |
| EC50 | 5.000 | 763.981 | 733.512 | 793.509 |
| EC60 | 5.253 | 790.895 | 761.295 | 823.236 |
| EC75 | 5.674 | 837.749 | 806.042 | 879.246 |
| EC80 | 5.842 | 857.104 | 823.417 | 903.725 |
| EC85 | 6.036 | 880.229 | 843.541 | 933.786 |
| EC90 | 6.282 | 910.214 | 868.84 | 973.843 |
| EC95 | 6.645 | 956.546 | 906.672 | 1037.62 |
| EC99 | 7.326 | 1049.91 | 980.01 | 1171.26 |




### 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: | 4/8/2025 | Test ID: | PpKCICR | Sample ID: | REF-Ref Toxicant |
| End Date: | 4/15/2025 | 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 | 0.6860 | 0.7130 | 0.7650 | 0.8180 |
| 300 | 0.9580 | 0.8190 | 0.8820 | 0.9680 |
| 450 | 0.8390 | 0.8270 | 0.8340 | 0.8450 |
| 600 | 0.6840 | 0.8010 | 0.7200 | 0.6950 |
| 750 | 0.4320 | 0.3830 | 0.4970 | 0.3910 |
| 900 | 0.1030 | 0.0940 | 0.1860 | 0.0690 |
| 1050 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

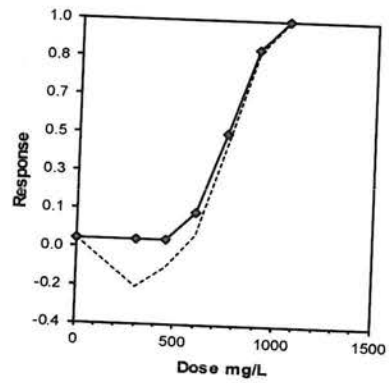
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| D-Control | 0.7455 | 1.0000 | 0.7455 | 0.6860 | 0.8180 | 7.834 | 4 | | | | 0.8295 | 1.0000 |
| 300 | 0.9068 | 1.2163 | 0.9068 | 0.8190 | 0.9680 | 7.717 | 4 | -4.317 | 2.290 | 0.0855 | 0.8295 | 1.0000 |
| 450 | 0.8363 | 1.1217 | 0.8363 | 0.8270 | 0.8450 | 0.913 | 4 | -2.430 | 2.290 | 0.0855 | 0.8295 | 1.0000 |
| 600 | 0.7250 | 0.9725 | 0.7250 | 0.6840 | 0.8010 | 7.291 | 4 | 0.549 | 2.290 | 0.0855 | 0.7250 | 0.8740 |
| 750 | 0.4258 | 0.5711 | 0.4258 | 0.3830 | 0.4970 | 12.243 | 4 | | | | 0.4258 | 0.5133 |
| 900 | 0.1130 | 0.1516 | 0.1130 | 0.0690 | 0.1860 | 44.910 | 4 | | | | 0.1130 | 0.1362 |
| 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) | 0.96269 | 0.844 | 0.11552 | -0.5275 |
| Bartlett's Test indicates equal variances (p = 0.04) | 8.17679 | 11.3449 | | |

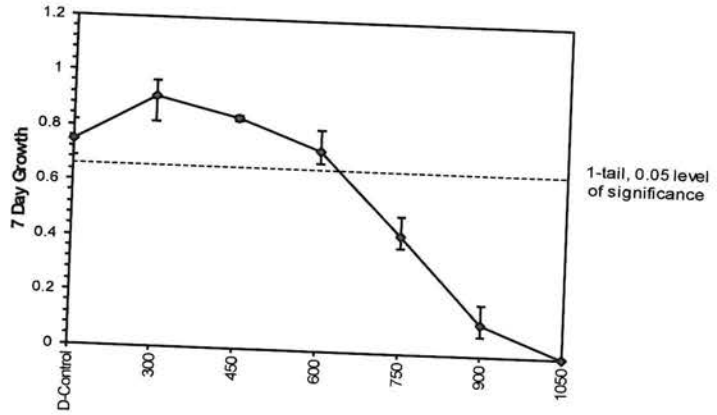
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 600 | >600 | | | 0.08553 | 0.11473 | 0.02835 | 0.00279 | 0.0013 | 3, 12 |
| Treatments vs D-Control | | | | | | | | | | |

### Linear Interpolation (200 Resamples)

| Point | mg/L | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 509.53 | 21.97 | 447.56 | 590.51 | 1.0186 |
| IC10 | 569.07 | 25.88 | 495.44 | 637.14 | -0.1101 |
| IC15 | 609.99 | 15.68 | 544.49 | 644.30 | -1.1123 |
| IC20 | 630.78 | 11.01 | 593.14 | 664.18 | -0.2799 |
| IC25 | 651.57 | 10.03 | 617.03 | 683.60 | -0.1012 |
| IC40 | 713.93 | 9.94 | 686.04 | 747.91 | 0.4850 |
| IC50 | 755.28 | 10.09 | 728.10 | 783.53 | 0.2280 |




### Dose-Response Plot



ETS
Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas***

PpKClCR Test Number: **127**

## *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.

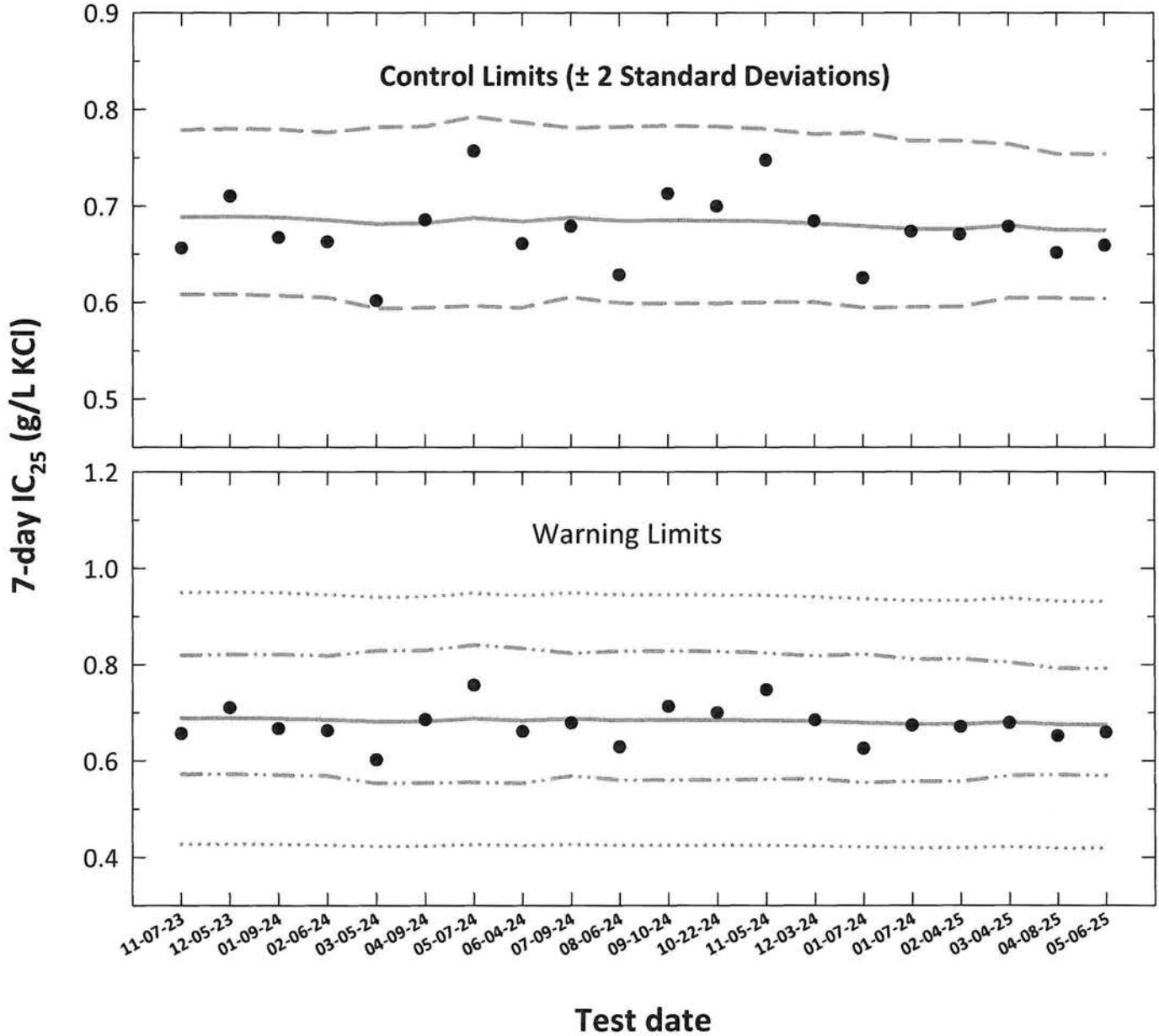
| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | Em N | Em N | Em N | Em N | Em N | N |
| Concentration | Parameter | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.89 | 7.83 | 7.84 | 7.78 | 7.86 | 7.58 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.1 | 8.0 | 8.2 | 8.0 |
| | Conductivity (µmhos/cm) | 297 | | 312 | | 306 | |
| | Alkalinity (mg $CaCO_3$/L) | 59 | | | | 59 | |
| | Hardness (mg $CaCO_3$/L) | 89 | | | | 69 | |
| | Temperature (°C) | 24.8 | 25.0 | 24.9 | 24.8 | 24.8 | 25.1 |
| 300 mg KCl/L | pH (S.U.) | 7.96 | 7.80 | 7.96 | 7.77 | 7.95 | 7.50 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.0 | 8.0 | 8.2 | 7.3 |
| | Conductivity (µmhos/cm) | 842 | | 850 | | 843 | |
| | Temperature (°C) | 24.8 | 24.6 | 24.8 | 24.7 | 24.7 | 24.8 |
| 450 mg KCl/L | pH (S.U.) | 7.98 | 7.80 | 7.96 | 7.76 | 7.95 | 7.52 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.0 | 8.0 | 8.2 | 7.3 |
| | Conductivity (µmhos/cm) | 1080 | | 1120 | | 1100 | |
| | Temperature (°C) | 24.9 | 24.6 | 24.8 | 24.7 | 24.7 | 24.8 |
| 600 mg KCl/L | pH (S.U.) | 7.99 | 7.80 | 7.96 | 7.76 | 7.96 | 7.53 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.1 | 8.0 | 8.2 | 7.2 |
| | Conductivity (µmhos/cm) | 1320 | | 1380 | | 1370 | |
| | Temperature (°C) | 24.9 | 24.8 | 24.8 | 24.8 | 24.7 | 25.0 |
| 750 mg KCl/L | pH (S.U.) | 7.99 | 7.80 | 7.97 | 7.75 | 7.96 | 7.53 |
| | Dissolved oxygen (mg/L) | 8.3 | 8.0 | 8.1 | 8.0 | 8.1 | 7.2 |
| | Conductivity (µmhos/cm) | 1610 | | 1660 | | 1650 | |
| | Temperature (°C) | 24.8 | 24.7 | 24.8 | 24.6 | 24.7 | 24.8 |
| 900 mg KCl/L | pH (S.U.) | 8.00 | 7.82 | 7.97 | 7.81 | 7.97 | 7.54 |
| | Dissolved oxygen (mg/L) | 8.4 | 8.0 | 8.1 | 8.0 | 8.2 | 7.1 |
| | Conductivity (µmhos/cm) | 1840 | | 1900 | | 1870 | |
| | Temperature (°C) | 24.8 | 24.7 | 24.8 | 24.6 | 24.7 | 24.7 |
| 1050 mg KCl/L | pH (S.U.) | 8.01 | 7.84 | 7.98 | 7.79 | 7.97 | 7.67 |
| | Dissolved oxygen (mg/L) | 8.4 | 7.9 | 8.1 | 8.0 | 8.2 | 7.1 |
| | Conductivity (µmhos/cm) | 2190 | | 2190 | | 2170 | |
| | Temperature (°C) | 24.8 | 24.7 | 24.9 | 24.6 | 24.7 | 24.7 |
| | | Initial | Final | Initial | Final | Initial | Final |

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **127**

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | VC | BSL | BSL | BSL | BSL | EM | EM | VC |
| Concentration | Parameter | | | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.89 | ~~7.87~~ 7.75 | 7.94 | 7.72 | 7.97 | 7.73 | 7.88 | 7.01 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.7 | 8.2 | 7.6 | 8.4 | 7.9 | 8.2 | 6.6 |
| | Conductivity (µmhos/cm) | 299 | | 294 | | 298 | | 316 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 58 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | 04-15-15 N | 83 | | | | 04-15-15 N | |
| | Temperature (°C) | 24.7 | 24.8 | 24.8 | 25.1 | 24.7 | 25.1 | 24.8 | 24.6 |
| 300 mg KCl/L | pH (S.U.) | 7.95 | 7.66 | 7.91 | 7.65 | 8.02 | 7.70 | 7.93 | 7.62 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.6 | 8.2 | 7.2 | 8.3 | 7.9 | 8.2 | 6.3 |
| | Conductivity (µmhos/cm) | 824 | | 828 | | 857 | | 850 | |
| | Temperature (°C) | 24.7 | 25.0 | 24.9 | 24.9 | 24.7 | 24.8 | 24.8 | 24.5 |
| 450 mg KCl/L | pH (S.U.) | 7.96 | 7.68 | 7.99 | 7.70 | 8.07 | 7.69 | 7.94 | 7.62 |
| | Dissolved oxygen (mg/L) | 8.0 | 7.6 | 8.3 | 7.2 | 8.3 | 8.0 | 8.2 | 6.5 |
| | Conductivity (µmhos/cm) | 1050 | | 1100 | | 1110 | | 1120 | |
| | Temperature (°C) | 24.8 | 25.1 | 25.0 | 24.9 | 24.8 | 24.9 | 24.8 | 24.5 |
| 600 mg KCl/L | pH (S.U.) | 7.96 | 7.67 | 8.00 | 7.73 | 8.06 | 7.67 | 7.93 | 7.62 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.5 | 8.2 | 7.3 | 8.3 | 8.0 | 8.2 | 6.5 |
| | Conductivity (µmhos/cm) | 1350 | | 1360 | | 1370 | | 1360 | |
| | Temperature (°C) | 24.7 | 24.7 | 24.8 | 24.9 | 24.8 | 24.9 | 24.8 | 24.5 |
| 750 mg KCl/L | pH (S.U.) | 7.96 | 7.67 | 8.01 | 7.74 | 8.07 | 7.73 | 7.94 | 7.62 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.6 | 8.2 | 7.4 | 8.2 | 8.0 | 8.2 | 6.4 |
| | Conductivity (µmhos/cm) | 1600 | | 1620 | | 1610 | | 1610 | |
| | Temperature (°C) | 24.9 | 24.7 | 24.8 | 25.0 | 24.8 | 24.9 | 24.7 | 24.7 |
| 900 mg KCl/L | pH (S.U.) | 7.99 | 7.77 | 8.02 | 7.74 | 8.07 | 7.77 | 7.95 | 7.62 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.7 | 8.3 | 7.3 | 8.3 | 8.0 | 8.2 | 6.3 |
| | Conductivity (µmhos/cm) | 1860 | | 1890 | | 1920 | | 1860 | |
| | Temperature (°C) | 24.9 | 24.9 | 24.8 | 24.8 | 24.9 | 25.0 | 24.7 | 24.6 |
| 1050 mg KCl/L | pH (S.U.) | 8.00 | 7.80 | 8.02 | 7.81 | 8.07 | 7.76 | 7.95 | 7.63 |
| | Dissolved oxygen (mg/L) | 8.1 | 7.8 | 8.3 | 7.6 | 8.2 | 8.0 | 8.2 | 6.7 |
| | Conductivity (µmhos/cm) | 2120 | | 2130 | | 2150 | | 2140 | |
| | Temperature (°C) | 24.7 | 24.7 | 24.9 | 25.0 | 24.9 | 24.8 | 24.7 | 24.6 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |

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




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

Entered and Reviewed by Jim Sumner

ETS
Environmental Testing Solutions, Inc.

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

| Test number | Test date | 7-day $IC_{25}$ ToxCal Determination (g/L KCl) | $Log_{10}$ Conversion | | | Anti-logarithmic Values (g/L KCl) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 7-day $IC_{25}$ | CT | S | CT | Control Limits | | Laboratory Calculated CV Warning Limits | | 75th Percentile CV Warning Limits | |
| | | | | | | | CT - 2S | CT + 2S | CT - 2CV | CT + 2CV | CT - $S_{A.75}$ | CT + $S_{A.75}$ |
| 1 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 06-04-24 | 0.6610 | -0.1798 | -0.1650 | 0.0303 | 0.6840 | 0.5949 | 0.7864 | 0.5537 | 0.8338 | 0.4241 | 0.9439 |
| 9 | 07-09-24 | 0.6789 | -0.1682 | -0.1624 | 0.0276 | 0.6880 | 0.6060 | 0.7811 | 0.5688 | 0.8233 | 0.4265 | 0.9494 |
| 10 | 08-06-24 | 0.6287 | -0.2015 | -0.1644 | 0.0289 | 0.6848 | 0.5994 | 0.7823 | 0.5602 | 0.8272 | 0.4246 | 0.9450 |
| 11 | 09-10-24 | 0.7128 | -0.1470 | -0.1642 | 0.0290 | 0.6852 | 0.5994 | 0.7832 | 0.5600 | 0.8283 | 0.4248 | 0.9456 |
| 12 | 10-22-24 | 0.6997 | -0.1551 | -0.1645 | 0.0289 | 0.6847 | 0.5994 | 0.7822 | 0.5601 | 0.8271 | 0.4245 | 0.9449 |
| 13 | 11-05-24 | 0.7473 | -0.1265 | -0.1648 | 0.0284 | 0.6842 | 0.6004 | 0.7797 | 0.5617 | 0.8238 | 0.4242 | 0.9442 |
| 14 | 12-03-24 | 0.6844 | -0.1647 | -0.1662 | 0.0276 | 0.6820 | 0.6006 | 0.7745 | 0.5626 | 0.8176 | 0.4228 | 0.9411 |
| 15 | 01-07-24 | 0.6255 | -0.2038 | -0.1680 | 0.0289 | 0.6792 | 0.5946 | 0.7757 | 0.5547 | 0.8213 | 0.4211 | 0.9372 |
| 16 | 01-07-24 | 0.6737 | -0.1715 | -0.1700 | 0.0276 | 0.6760 | 0.5955 | 0.7675 | 0.5569 | 0.8113 | 0.4191 | 0.9329 |
| 17 | 02-04-25 | 0.6706 | -0.1735 | -0.1699 | 0.0275 | 0.6762 | 0.5956 | 0.7676 | 0.5571 | 0.8114 | 0.4192 | 0.9331 |
| 18 | 03-04-25 | 0.6786 | -0.1684 | -0.1676 | 0.0254 | 0.6798 | 0.6047 | 0.7643 | 0.5693 | 0.8041 | 0.4215 | 0.9381 |
| 19 | 04-08-25 | 0.6516 | -0.1860 | -0.1705 | 0.0239 | 0.6753 | 0.6048 | 0.7540 | 0.5708 | 0.7919 | 0.4187 | 0.9319 |
| 20 | 05-06-25 | 0.6589 | -0.1812 | -0.1709 | 0.0241 | 0.6746 | 0.6039 | 0.7537 | 0.5697 | 0.7918 | 0.4183 | 0.9310 |

*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.

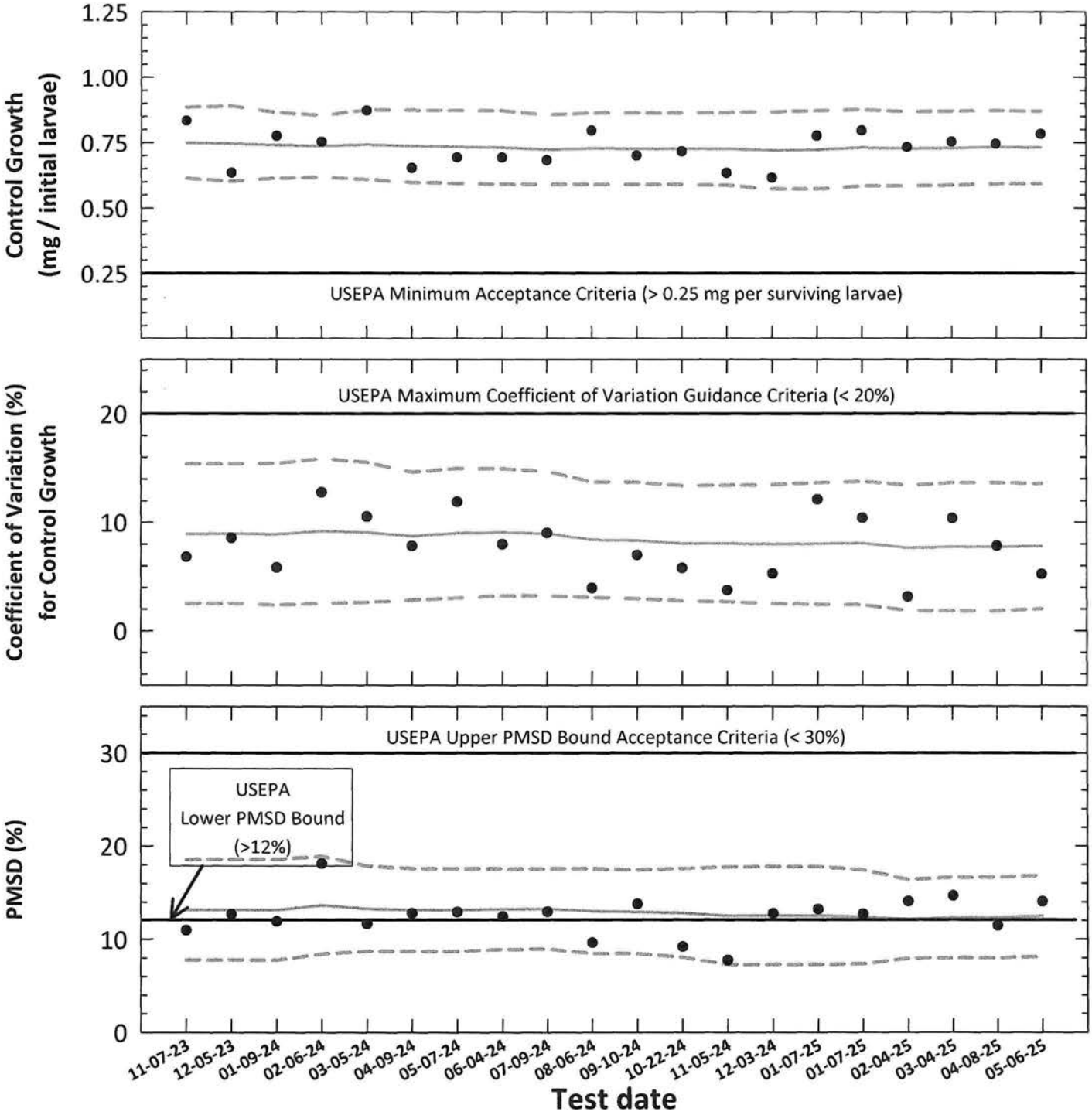
$S_{A.75}$ = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

**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 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 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 |
| 8 | 06-04-24 | 100 | 0.693 | 8.0 | 0.0862 | 12.4 | 0.732 | 0.591 | 0.872 | 9.1 | 3.2 | 14.9 | 13.2 | 8.9 | 17.5 |
| 9 | 07-09-24 | 100 | 0.683 | 9.0 | 0.0883 | 12.9 | 0.724 | 0.591 | 0.857 | 9.0 | 3.2 | 14.7 | 13.2 | 8.9 | 17.5 |
| 10 | 08-06-24 | 100 | 0.796 | 3.9 | 0.0766 | 9.6 | 0.728 | 0.591 | 0.865 | 8.4 | 3.0 | 13.7 | 13.0 | 8.4 | 17.5 |
| 11 | 09-10-24 | 100 | 0.701 | 7.0 | 0.0964 | 13.8 | 0.728 | 0.591 | 0.865 | 8.3 | 2.9 | 13.7 | 12.9 | 8.5 | 17.4 |
| 12 | 10-22-24 | 100 | 0.717 | 5.8 | 0.0659 | 9.2 | 0.727 | 0.590 | 0.864 | 8.1 | 2.7 | 13.4 | 12.8 | 8.0 | 17.6 |
| 13 | 11-05-24 | 100 | 0.635 | 3.7 | 0.0491 | 7.7 | 0.727 | 0.588 | 0.866 | 8.0 | 2.7 | 13.4 | 12.5 | 7.3 | 17.8 |
| 14 | 12-03-24 | 100 | 0.615 | 5.3 | 0.0785 | 12.8 | 0.721 | 0.573 | 0.868 | 8.0 | 2.5 | 13.5 | 12.5 | 7.3 | 17.8 |
| 15 | 01-07-25 | 100 | 0.776 | 12.1 | 0.1024 | 13.2 | 0.723 | 0.573 | 0.872 | 8.0 | 2.4 | 13.7 | 12.5 | 7.3 | 17.8 |
| 16 | 01-07-25 | 100 | 0.796 | 10.4 | 0.1010 | 12.7 | 0.731 | 0.585 | 0.877 | 8.1 | 2.4 | 13.8 | 12.4 | 7.3 | 17.4 |
| 17 | 02-04-25 | 100 | 0.733 | 3.1 | 0.1030 | 14.1 | 0.727 | 0.585 | 0.869 | 7.6 | 1.8 | 13.4 | 12.2 | 7.9 | 16.4 |
| 18 | 03-04-25 | 100 | 0.754 | 10.4 | 0.1103 | 14.6 | 0.729 | 0.588 | 0.871 | 7.7 | 1.8 | 13.6 | 12.3 | 8.0 | 16.7 |
| 19 | 04-08-25 | 100 | 0.746 | 7.8 | 0.0855 | 11.5 | 0.733 | 0.593 | 0.873 | 7.7 | 1.8 | 13.7 | 12.3 | 8.0 | 16.7 |
| 20 | 05-06-25 | 100 | 0.783 | 5.2 | 0.1099 | 14.0 | 0.732 | 0.593 | 0.871 | 7.8 | 2.0 | 13.6 | 12.5 | 8.1 | 16.8 |

*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: **128**

| ***Dilution preparation information:*** | | | | | | | ***Comments:*** |
|---|---|---|---|---|---|---|---|
| KCl Stock INSS number: | | | INSS 7376 | | | | |
| 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: 04-29-25 | ~~05-06-25 1450 TO 05-06-25 0455~~ | *Artemia* CHM number: | CHM1385 |
| Hatch dates and times: | 05-05-25 1450 TO 05-06-25 0455 | ***Drying information for weight determination:*** | |
| Transfer vessel information: | pH = ~~24.2~~ 8.06 S.U. Temperature = ~~8.06~~ °C 24.2°C | Date / Time in oven: | 05-13-25 0605 |
| | | *Initial oven temperature: | 60 °C |
| Average transfer volume: | < 0.25 mL | Date / Time out of oven: | 05-14-25 0605 |
| | | *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 | 05-06-25 | 0500 | H | 1400 | H | 0708 | H | 04-30-25 B |
| 1 | 05-07-25 | 0500 | H | 1420 | H | 0710 | H | ↓ |
| 2 | 05-08-25 | 0500 | H | 1330 | H | 0710 | H | 04-30-25 C |
| 3 | 05-09-25 | 0500 | H | 1100 | H | 0700 | H | ↓ |
| 4 | 05-10-25 | 0600 | H | 1200 | H | 0800 | H | 05-08-25 A |
| 5 | 05-11-25 | 0600 | H | 1200 | H | 0800 | H | ↓ |
| 6 | 05-12-25 | 0500 | H | 1100 | H | 0700 | H | ↓ |
| 7 | 05-13-25 | | | | | 0520 | H | |

***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) | 820.7 |
| Average weight per initial larvae: | 0.783 | | **NOEC** (mg/L KCl) | 600 |
| Average weight per surviving larvae: | 0.783 | ≥ 0.25 mg/larvae | **LOEC** (mg/L KCl) | 750 |
| | | | **ChV** (mg/L KCl) | 670.8 |
| | | | **$IC_{25}$** (mg/L KCl) | 658.9 |

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **128**

***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 16 | 10 | 10 | 10 | 10 | 10 | 10 |
| *A = Pan weight (mg)<br>Tray color code: Turquoise<br>Analyst: XL<br>Date: 04-22-25 | 12.82 | 13.60 | 14.28 | 14.42 | 13.81 | 12.88 | 13.59 | 12.00 | 13.35 | 12.80 | 14.97 | 14.51 |
| *B = Pan + Larvae weight (mg)<br>Analyst: XL<br>Date: 05-15-25 | 20.14 | 21.32 | 22.57 | 22.39 | 23.63 | 23.58 | 22.89 | 20.00 | 20.69 | 20.65 | 19.24 | 21.59 |
| C = Larvae weight (mg) = B – A<br>Analyst: JL | 7.32 | 7.72 | 8.29 | 7.97 | 9.82 | 10.70 | 9.30 | 8.00 | 7.34 | 7.85 | 7.27 | 7.08 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JL | 0.732 | 0.772 | 0.829 | 0.797 | 0.982 | 1.070 | 0.930 | 0.800 | 0.734 | 0.785 | 0.727 | 0.708 |
| Average weight per initial number of larvae (mg) / Percent reduction from control (%) | 0.783 | | | | 0.946 | | - 20.87. | | 0.739 | | 5.67. | |

*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:*** |
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Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **128**

***Survival and Growth Data***

| Day | 600 mg KCl/L | | | | 750 mg KCl/L | | | | 900 mg KCl/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 9 1d | 9 1d | 9 1d | 6 4d | 6 4d | 6 4d | 7 3d |
| 2 | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 9 | 5 1d | 5 1d | 6 | 6 1d |
| 3 | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 9 | 5 | 5 | 6 | 6 |
| 4 | 10 | 10 | 10 | 10 | 8 2d | 8 1d | 9 | 9 | 4 1d | 5 | 2 4d | 6 |
| 5 | 10 | 10 | 9 1d | 10 | 7 1d | 8 | 9 | 9 | 3 1d | 4 1d | 2 | 4 2d |
| 6 | 10 | 10 | 9 | 10 | 7 | 8 | 8 1d | 7 2d | 3 | 3 1d | 2 | 3 1d |
| 7 | 10 | 10 | 9 | 10 | 7 | 7 1d | 8 | 7 | 2 1d | 2 1d | 2 | 3 |
| *A = Pan weight (mg)<br>Tray color code: Turquoise<br>Analyst: XL<br>Date: 04-22-25 | 13.82 | 13.31 | 13.90 | 13.32 | 13.39 | 12.94 | 13.50 | 12.32 | 14.24 | 13.89 | 15.54 | 12.75 |
| *B = Pan + Larvae weight (mg)<br>Analyst: XL<br>Date: 05-15-25 | 20.96 | 21.33 | 21.15 | 20.05 | 18.37 | 18.10 | 19.25 | 17.37 | 15.83 | 15.40 | 16.99 | 15.06 |
| C = Larvae weight (mg) = B – A<br>Analyst: [illegible] | 7.14 | 8.02 | 7.25 | 6.73 | 4.98 | 5.16 | 5.75 | 5.05 | 1.59 | 1.51 | 1.45 | 2.31 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: [illegible] | 0.714 | 0.802 | 0.725 | 0.673 | 0.498 | 0.516 | 0.575 | 0.505 | 0.159 | 0.151 | 0.145 | 0.231 |
| Average weight per initial number of larvae (mg) | 0.729 | | | | 0.524 | | | | 0.172 | | | |
| Percent reduction from control (%) | | | 6.9% | | | | 33.1% | | | | 78.1% | |

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

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

***Comments:***

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **128**

***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 | 5 5d |
| 2 | 2 2d | 5 | 2 2d | 5 |
| 3 | 2 | 3 2d | 2 | 3 2d |
| 4 | 2 | 3 | 2 | 2 1d |
| 5 | 2 | 1 2d | 1 1d | 2 |
| 6 | 2 | 0 1d | 1 | 2 |
| 7 | 1 1d | 0 | 1 | 2 |
| *A = Pan weight (mg)<br>Tray color code: Turquoise<br>Analyst: XL<br>Date: 04-22-25 | 12.76 | 12.09 | 13.15 | 13.65 |
| *B = Pan + Larvae weight (mg)<br>Analyst: XL<br>Date: 05-15-25 | 13.65 | 12.09 | 13.91 | 15.47 |
| C = Larvae weight (mg) = B – A<br>Analyst: JL | 0.89 | 05-15-25 JL | 0.76 | 1.82 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JL | 0.089 | 0 | 0.076 | 0.182 |
| Average weight per initial number of larvae (mg) | 0.087 | | | |
| Percent reduction from control (%) | | | 88.9% | |

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

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

| ***Comments:*** |
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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: 128

Test dates: May 06-13, 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 | 12.82 | 20.14 | 7.32 | 0.732 | 0.783 | 5.2 | 0.732 | 100.0 | 0.783 | 5.2 | Not applicable |
| | B | 10 | 10 | 13.60 | 21.32 | 7.72 | 0.772 | | | 0.772 | | | | |
| | C | 10 | 10 | 14.28 | 22.57 | 8.29 | 0.829 | | | 0.829 | | | | |
| | D | 10 | 10 | 14.42 | 22.39 | 7.97 | 0.797 | | | 0.797 | | | | |
| 300 | E | 10 | 10 | 13.81 | 23.63 | 9.82 | 0.982 | 0.946 | 11.9 | 0.982 | 100.0 | 0.946 | 11.9 | -20.8 |
| | F | 10 | 10 | 12.88 | 23.58 | 10.70 | 1.070 | | | 1.070 | | | | |
| | G | 10 | 10 | 13.59 | 22.89 | 9.30 | 0.930 | | | 0.930 | | | | |
| | H | 10 | 10 | 12.00 | 20.00 | 8.00 | 0.800 | | | 0.800 | | | | |
| 450 | I | 10 | 10 | 13.35 | 20.69 | 7.34 | 0.734 | 0.739 | 4.5 | 0.734 | 100.0 | 0.739 | 4.5 | 5.6 |
| | J | 10 | 10 | 12.80 | 20.65 | 7.85 | 0.785 | | | 0.785 | | | | |
| | K | 10 | 10 | 11.97 | 19.24 | 7.27 | 0.727 | | | 0.727 | | | | |
| | L | 10 | 10 | 14.51 | 21.59 | 7.08 | 0.708 | | | 0.708 | | | | |
| 600 | M | 10 | 10 | 13.82 | 20.96 | 7.14 | 0.714 | 0.749 | 8.8 | 0.714 | 97.5 | 0.729 | 7.4 | 6.9 |
| | N | 10 | 10 | 13.31 | 21.33 | 8.02 | 0.802 | | | 0.802 | | | | |
| | O | 10 | 9 | 13.90 | 21.15 | 7.25 | 0.806 | | | 0.725 | | | | |
| | P | 10 | 10 | 13.32 | 20.05 | 6.73 | 0.673 | | | 0.673 | | | | |
| 750 | Q | 10 | 7 | 13.39 | 18.37 | 4.98 | 0.711 | 0.722 | 1.5 | 0.498 | 72.5 | 0.524 | 6.7 | 33.1 |
| | R | 10 | 7 | 12.94 | 18.10 | 5.16 | 0.737 | | | 0.516 | | | | |
| | S | 10 | 8 | 13.50 | 19.25 | 5.75 | 0.719 | | | 0.575 | | | | |
| | T | 10 | 7 | 12.32 | 17.37 | 5.05 | 0.721 | | | 0.505 | | | | |
| 900 | U | 10 | 2 | 14.24 | 15.83 | 1.59 | 0.795 | 0.761 | 3.8 | 0.159 | 22.5 | 0.172 | 23.4 | 78.1 |
| | V | 10 | 2 | 13.89 | 15.40 | 1.51 | 0.755 | | | 0.151 | | | | |
| | W | 10 | 2 | 15.54 | 16.99 | 1.45 | 0.725 | | | 0.145 | | | | |
| | X | 10 | 3 | 12.75 | 15.06 | 2.31 | 0.770 | | | 0.231 | | | | |
| 1050 | Y | 10 | 1 | 12.76 | 13.65 | 0.89 | 0.890 | 0.853 | 9.5 | 0.089 | 10.0 | 0.087 | 86.1 | 88.9 |
| | Z | 10 | 0 | 0.00 | 0.00 | 0.00 | 0.000 | | | 0.000 | | | | |
| | AA | 10 | 1 | 13.15 | 13.91 | 0.76 | 0.760 | | | 0.076 | | | | |
| | BB | 10 | 2 | 13.65 | 15.47 | 1.82 | 0.910 | | | 0.182 | | | | |

**Dunnett's MSD value:** 0.1099 — MSD = Minimum Significant Difference

**PMSD:** 14.0 — PMSD = Percent Minimum Significant Difference

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

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

Entered and Reviewed by Jim Sumner

Environmental Testing Solutions, Inc.

# Statistical Analyses

Entered and Reviewed by Jim Sumner

## Larval Fish Growth and Survival Test-7 Day Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 5/6/2025 | Test ID: | PpKCICR | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/13/2025 | 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 | 1.0000 | 0.9000 | 1.0000 |
| 750 | 0.7000 | 0.7000 | 0.8000 | 0.7000 |
| 900 | 0.2000 | 0.2000 | 0.2000 | 0.3000 |
| 1050 | 0.1000 | 0.0000 | 0.1000 | 0.2000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | | | 0 | 40 |
| 300 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | 18.00 | 10.00 | 0 | 40 |
| 450 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | 18.00 | 10.00 | 0 | 40 |
| 600 | 0.9750 | 0.9750 | 1.3713 | 1.2490 | 1.4120 | 5.942 | 4 | 16.00 | 10.00 | 1 | 40 |
| *750 | 0.7250 | 0.7250 | 1.0202 | 0.9912 | 1.1071 | 5.685 | 4 | 10.00 | 10.00 | 11 | 40 |
| *900 | 0.2250 | 0.2250 | 0.4926 | 0.4636 | 0.5796 | 11.772 | 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 |

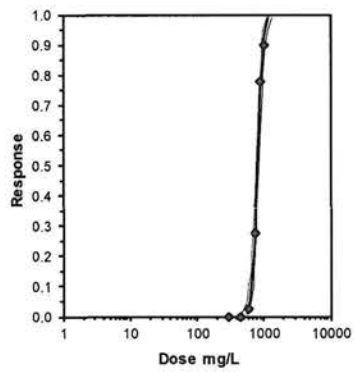
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.85011 | 0.896 | -0.2603 | 3.08946 |
| Equality of variance cannot be confirmed | | | | |

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

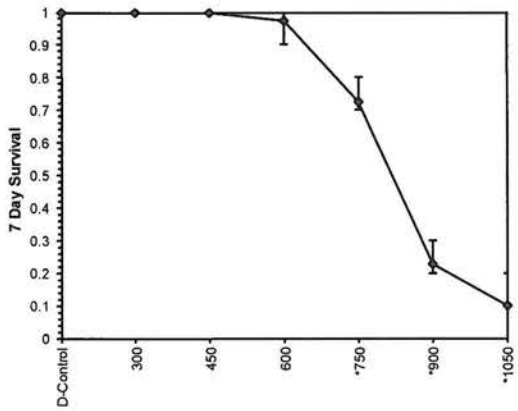
Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 13.8975 | 1.77642 | 10.4157 | 17.3793 | 0 | 1.51336 | 9.48773 | 0.82427 | 2.91419 | 0.07196 | 3 |
| Intercept | -35.5 | 5.18715 | -45.667 | -25.333 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 558.21 | 484.907 | 609.33 |
| EC05 | 3.355 | 624.935 | 562.136 | 668.824 |
| EC10 | 3.718 | 663.706 | 607.652 | 703.531 |
| EC15 | 3.964 | 691.216 | 640.016 | 728.426 |
| EC20 | 4.158 | 713.89 | 666.597 | 749.247 |
| EC25 | 4.326 | 733.935 | 689.916 | 767.99 |
| EC40 | 4.747 | 786.975 | 749.927 | 819.971 |
| EC50 | 5.000 | 820.712 | 786.029 | 855.618 |
| EC60 | 5.253 | 855.895 | 821.48 | 895.412 |
| EC75 | 5.674 | 917.749 | 878.793 | 971.401 |
| EC80 | 5.842 | 943.517 | 901.239 | 1004.87 |
| EC85 | 6.036 | 974.468 | 927.421 | 1046.13 |
| EC90 | 6.282 | 1014.86 | 960.628 | 1101.4 |
| EC95 | 6.645 | 1077.82 | 1010.86 | 1190.12 |
| EC99 | 7.326 | 1206.66 | 1109.97 | 1379.16 |




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: | 5/6/2025 | Test ID: | PpKCICR | Sample ID: | REF-Ref Toxicant |
| End Date: | 5/13/2025 | 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 | 0.7320 | 0.7720 | 0.8290 | 0.7970 |
| 300 | 0.9820 | 1.0700 | 0.9300 | 0.8000 |
| 450 | 0.7340 | 0.7850 | 0.7270 | 0.7080 |
| 600 | 0.7140 | 0.8020 | 0.7250 | 0.6730 |
| 750 | 0.4980 | 0.5160 | 0.5750 | 0.5050 |
| 900 | 0.1590 | 0.1510 | 0.1450 | 0.2310 |
| 1050 | 0.0890 | 0.0000 | 0.0760 | 0.1820 |

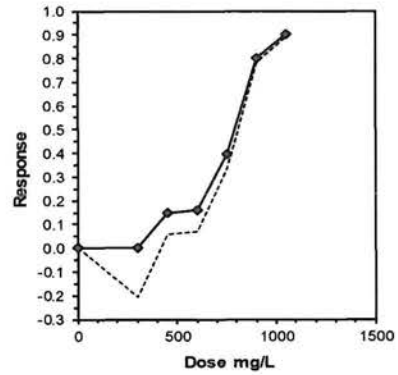
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| D-Control | 0.7825 | 1.0000 | 0.7825 | 0.7320 | 0.8290 | 5.234 | 4 | | | | 0.8640 | 1.0000 |
| 300 | 0.9455 | 1.2083 | 0.9455 | 0.8000 | 1.0700 | 11.941 | 4 | -3.398 | 2.290 | 0.1099 | 0.8640 | 1.0000 |
| 450 | 0.7385 | 0.9438 | 0.7385 | 0.7080 | 0.7850 | 4.453 | 4 | 0.917 | 2.290 | 0.1099 | 0.7385 | 0.8547 |
| 600 | 0.7285 | 0.9310 | 0.7285 | 0.6730 | 0.8020 | 7.394 | 4 | 1.126 | 2.290 | 0.1099 | 0.7285 | 0.8432 |
| 750 | 0.5235 | 0.6690 | 0.5235 | 0.4980 | 0.5750 | 6.709 | 4 | | | | 0.5235 | 0.6059 |
| 900 | 0.1715 | 0.2192 | 0.1715 | 0.1450 | 0.2310 | 23.370 | 4 | | | | 0.1715 | 0.1985 |
| 1050 | 0.0868 | 0.1109 | 0.0868 | 0.0000 | 0.1820 | 86.054 | 4 | | | | 0.0868 | 0.1004 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.95262 | 0.844 | -0.2859 | 1.77288 |
| Bartlett's Test indicates equal variances (p = 0.17) | 5.00374 | 11.3449 | | |

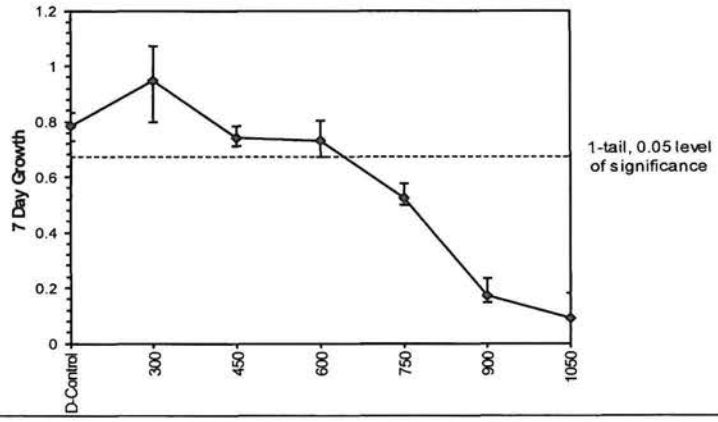
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 600 | >600 | | | 0.10985 | 0.14038 | 0.04049 | 0.0046 | 0.00234 | 3, 12 |
| Treatments vs D-Control | | | | | | | | | | |

### Linear Interpolation (200 Resamples)

| Point | mg/L | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 351.63 | 15.37 | 331.77 | 433.99 | 1.5270 |
| IC10 | 403.27 | 60.76 | 363.53 | 731.52 | 2.2859 |
| IC15 | 511.50 | 82.77 | 361.34 | 710.34 | -0.0720 |
| IC20 | 627.29 | 36.34 | 458.99 | 684.44 | -2.2159 |
| IC25 | 658.90 | 18.47 | 597.16 | 713.45 | -0.1684 |
| IC40 | 752.17 | 11.27 | 709.80 | 778.26 | -0.4903 |
| IC50 | 788.99 | 7.45 | 766.92 | 812.24 | -0.0044 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **128**

## *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.

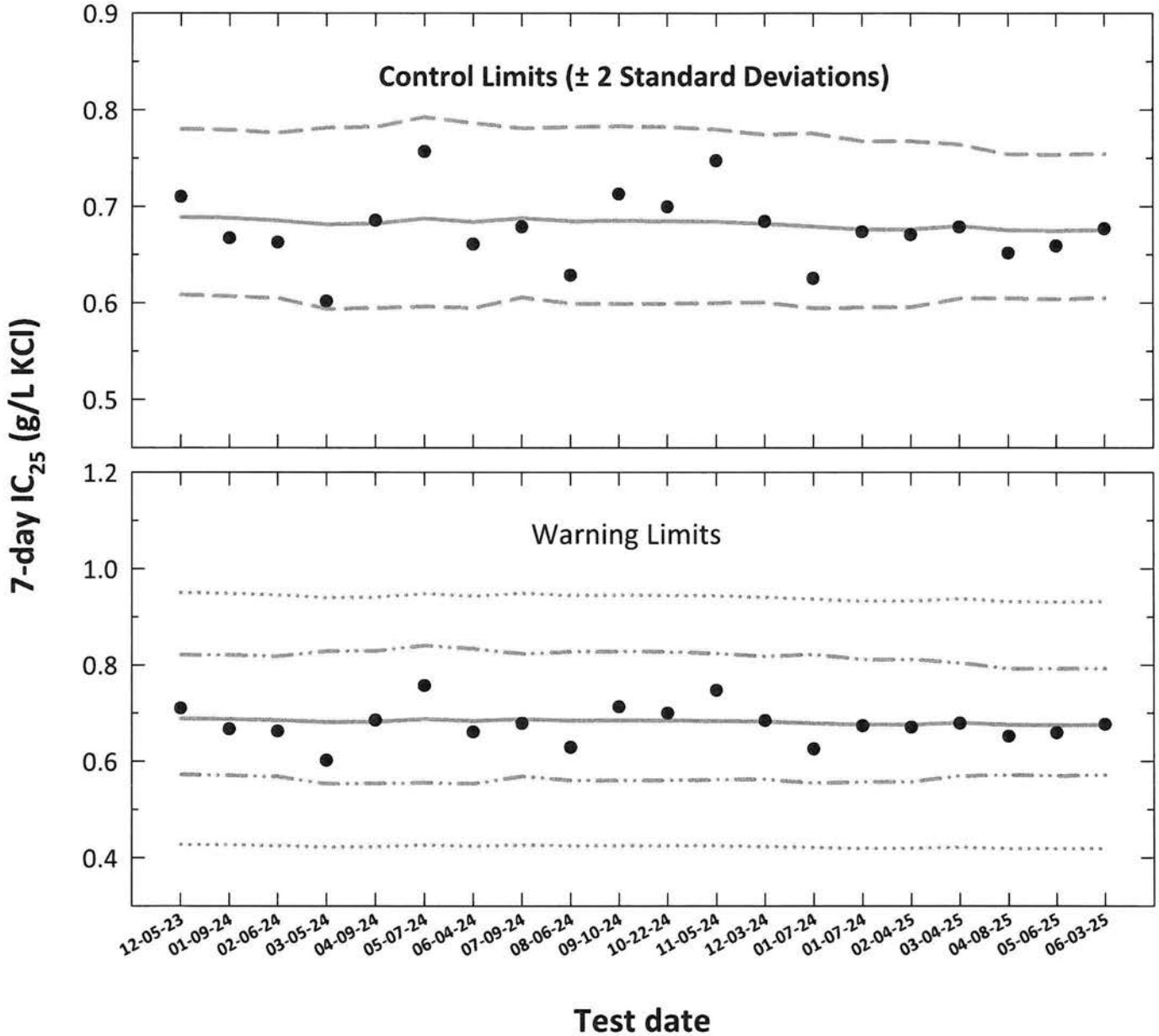
| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | N | Em | Em | Em | Em | N |
| Concentration | Parameter | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 8.13 | 7.78 | 7.80 | 7.76 | 7.81 | 7.56 |
| | Dissolved oxygen (mg/L) | 8.2 | 8.0 | 8.0 | 7.9 | 8.0 | 6.4 |
| | Conductivity (µmhos/cm) | 296 | | 313 | | 300 | |
| | Alkalinity (mg $CaCO_3$/L) | 62 | | | | 60 | |
| | Hardness (mg $CaCO_3$/L) | 88 | | | N 05-N-15 H | 86 | |
| | Temperature (°C) | 24.8 | 25.0 | 24.7 | 24.7 | 24.7 | 24.3 |
| 300 mg KCl/L | pH (S.U.) | 8.11 | 7.74 | 7.90 | 7.70 | 7.90 | 7.47 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 8.0 | 7.9 | 8.0 | 5.9 |
| | Conductivity (µmhos/cm) | 848 | | 846 | | 850 | |
| | Temperature (°C) | 24.7 | 25.2 | 24.7 | 24.6 | 24.7 | 24.6 |
| 450 mg KCl/L | pH (S.U.) | 8.11 | 7.75 | 7.91 | 7.72 | 7.90 | 7.45 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.0 | 7.9 | 8.0 | 5.8 |
| | Conductivity (µmhos/cm) | 1090 | | 1130 | | 1130 | |
| | Temperature (°C) | 24.8 | 24.8 | 24.6 | 24.6 | 24.6 | 24.6 |
| 600 mg KCl/L | pH (S.U.) | 8.11 | 7.75 | 7.91 | 7.69 | 7.90 | 7.45 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.0 | 7.8 | 8.1 | 5.8 |
| | Conductivity (µmhos/cm) | 1330 | | 1370 | | 1370 | |
| | Temperature (°C) | 24.8 | 24.8 | 24.6 | 24.6 | 24.7 | 24.4 |
| 750 mg KCl/L | pH (S.U.) | 8.11 | 7.75 | 7.91 | 7.69 | 7.90 | 7.44 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.0 | 7.8 | 8.1 | 6.0 |
| | Conductivity (µmhos/cm) | 1620 | | 1630 | | 1630 | |
| | Temperature (°C) | 24.7 | 24.8 | 24.6 | 24.4 | 24.6 | 24.4 |
| 900 mg KCl/L | pH (S.U.) | 8.11 | 7.73 | 7.92 | 7.71 | 7.90 | 7.47 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.0 | 7.8 | 8.1 | 6.5 |
| | Conductivity (µmhos/cm) | 1880 | | 1880 | | 1880 | |
| | Temperature (°C) | 24.7 | 25.2 | 24.7 | 24.4 | 24.6 | 24.4 |
| 1050 mg KCl/L | pH (S.U.) | 8.12 | 7.74 | 7.92 | 7.72 | 7.91 | 7.52 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.0 | 7.8 | 8.1 | 6.2 |
| | Conductivity (µmhos/cm) | 2150 | | 2150 | | 2170 | |
| | Temperature (°C) | 24.9 | 25.1 | 24.7 | 24.5 | 24.6 | 24.3 |
| | | Initial | Final | Initial | Final | Initial | Final |

ETS
Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **128**

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | N | BSL | BSL | BSL | BSL | KL | XL | N |
| Concentration | Parameter | | | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 8.01 | 7.74 | 7.92 | 7.64 | 7.97 | 7.73 | 8.00 | 7.46 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.4 | 7.4 | 8.4 | 7.9 | 8.3 | 6.2 |
| | Conductivity (µmhos/cm) | 295 | | 298 | | 296 | | 309 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 59 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | 05-19-15 M | 88 | | | | | 05-19-15 M |
| | Temperature (°C) | 24.7 | 24.8 | 24.8 | 25.0 | 24.7 | 24.9 | 24.8 | 24.6 |
| 300 mg KCl/L | pH (S.U.) | 7.97 | 7.74 | 8.01 | 7.61 | 8.01 | 7.72 | 7.90 | 7.37 |
| | Dissolved oxygen (mg/L) | 8.3 | 7.9 | 8.4 | 7.1 | 8.4 | 7.8 | 8.3 | 5.8 |
| | Conductivity (µmhos/cm) | 806 | | 814 | | 834 | | 858 | |
| | Temperature (°C) | 24.6 | 24.9 | 24.8 | 24.7 | 24.7 | 24.7 | 24.8 | 24.6 |
| 450 mg KCl/L | pH (S.U.) | 7.96 | 7.74 | 8.01 | 7.61 | 8.06 | 7.72 | 7.93 | 7.37 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 8.4 | 7.0 | 8.3 | 7.8 | 8.3 | 6.1 |
| | Conductivity (µmhos/cm) | 1060 | | 1080 | | 1060 | | 1090 | |
| | Temperature (°C) | 24.6 | 24.6 | 24.7 | 24.7 | 24.8 | 24.6 | 24.7 | 24.3 |
| 600 mg KCl/L | pH (S.U.) | 7.97 | 7.75 | 8.03 | 7.64 | 8.06 | 7.71 | 7.95 | 7.39 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 8.4 | 7.3 | 8.3 | 7.7 | 8.3 | 6.0 |
| | Conductivity (µmhos/cm) | 1310 | | 1340 | | 1310 | | 1350 | |
| | Temperature (°C) | 24.6 | 24.6 | 24.9 | 24.6 | 24.8 | 24.8 | 24.7 | 24.7 |
| 750 mg KCl/L | pH (S.U.) | 7.97 | 7.70 | 8.03 | 7.63 | 8.07 | 7.71 | 7.95 | 7.43 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.9 | 8.4 | 7.2 | 8.3 | 7.6 | 8.3 | 6.3 |
| | Conductivity (µmhos/cm) | 1560 | | 1570 | | 1570 | | 1590 | |
| | Temperature (°C) | 24.7 | 24.9 | 24.7 | 24.9 | 24.9 | 24.7 | 24.8 | 24.4 |
| 900 mg KCl/L | pH (S.U.) | 7.98 | 7.73 | 8.03 | 7.67 | 8.08 | 7.78 | 7.96 | 7.43 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.7 | 8.4 | 7.2 | 8.3 | 7.9 | 8.3 | 5.8 |
| | Conductivity (µmhos/cm) | 1800 | | 1820 | | 1810 | | 1840 | |
| | Temperature (°C) | 24.7 | 24.7 | 24.7 | 24.6 | 24.8 | 24.7 | 24.8 | 24.4 |
| 1050 mg KCl/L | pH (S.U.) | 7.98 | 7.74 | 7.78 | 7.64 | 8.07 | 7.78 | 7.97 | 7.43 |
| | Dissolved oxygen (mg/L) | 8.2 | 7.8 | 8.4 | 7.1 | 8.4 | 7.9 | 8.3 | 6.1 |
| | Conductivity (µmhos/cm) | 2070 | | 2030 | | 2030 | | 2130 | |
| | Temperature (°C) | 24.8 | 24.8 | 24.7 | 24.6 | 24.8 | 24.7 | 24.9 | 24.4 |
| | | 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 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 06-04-24 | 0.6610 | -0.1798 | -0.1650 | 0.0303 | 0.6840 | 0.5949 | 0.7864 | 0.5537 | 0.8338 | 0.4241 | 0.9439 |
| 8 | 07-09-24 | 0.6789 | -0.1682 | -0.1624 | 0.0276 | 0.6880 | 0.6060 | 0.7811 | 0.5688 | 0.8233 | 0.4265 | 0.9494 |
| 9 | 08-06-24 | 0.6287 | -0.2015 | -0.1644 | 0.0289 | 0.6848 | 0.5994 | 0.7823 | 0.5602 | 0.8272 | 0.4246 | 0.9450 |
| 10 | 09-10-24 | 0.7128 | -0.1470 | -0.1642 | 0.0290 | 0.6852 | 0.5994 | 0.7832 | 0.5600 | 0.8283 | 0.4248 | 0.9456 |
| 11 | 10-22-24 | 0.6997 | -0.1551 | -0.1645 | 0.0289 | 0.6847 | 0.5994 | 0.7822 | 0.5601 | 0.8271 | 0.4245 | 0.9449 |
| 12 | 11-05-24 | 0.7473 | -0.1265 | -0.1648 | 0.0284 | 0.6842 | 0.6004 | 0.7797 | 0.5617 | 0.8238 | 0.4242 | 0.9442 |
| 13 | 12-03-24 | 0.6844 | -0.1647 | -0.1662 | 0.0276 | 0.6820 | 0.6006 | 0.7745 | 0.5626 | 0.8176 | 0.4228 | 0.9411 |
| 14 | 01-07-24 | 0.6255 | -0.2038 | -0.1680 | 0.0289 | 0.6792 | 0.5946 | 0.7757 | 0.5547 | 0.8213 | 0.4211 | 0.9372 |
| 15 | 01-07-24 | 0.6737 | -0.1715 | -0.1700 | 0.0276 | 0.6760 | 0.5955 | 0.7675 | 0.5569 | 0.8113 | 0.4191 | 0.9329 |
| 16 | 02-04-25 | 0.6706 | -0.1735 | -0.1699 | 0.0275 | 0.6762 | 0.5956 | 0.7676 | 0.5571 | 0.8114 | 0.4192 | 0.9331 |
| 17 | 03-04-25 | 0.6786 | -0.1684 | -0.1676 | 0.0254 | 0.6798 | 0.6047 | 0.7643 | 0.5693 | 0.8041 | 0.4215 | 0.9381 |
| 18 | 04-08-25 | 0.6516 | -0.1860 | -0.1705 | 0.0239 | 0.6753 | 0.6048 | 0.7540 | 0.5708 | 0.7919 | 0.4187 | 0.9319 |
| 19 | 05-06-25 | 0.6589 | -0.1812 | -0.1709 | 0.0241 | 0.6746 | 0.6039 | 0.7537 | 0.5697 | 0.7918 | 0.4183 | 0.9310 |
| 20 | 06-03-25 | 0.6768 | -0.1695 | -0.1703 | 0.0239 | 0.6757 | 0.6053 | 0.7543 | 0.5715 | 0.7920 | 0.4189 | 0.9324 |

*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.

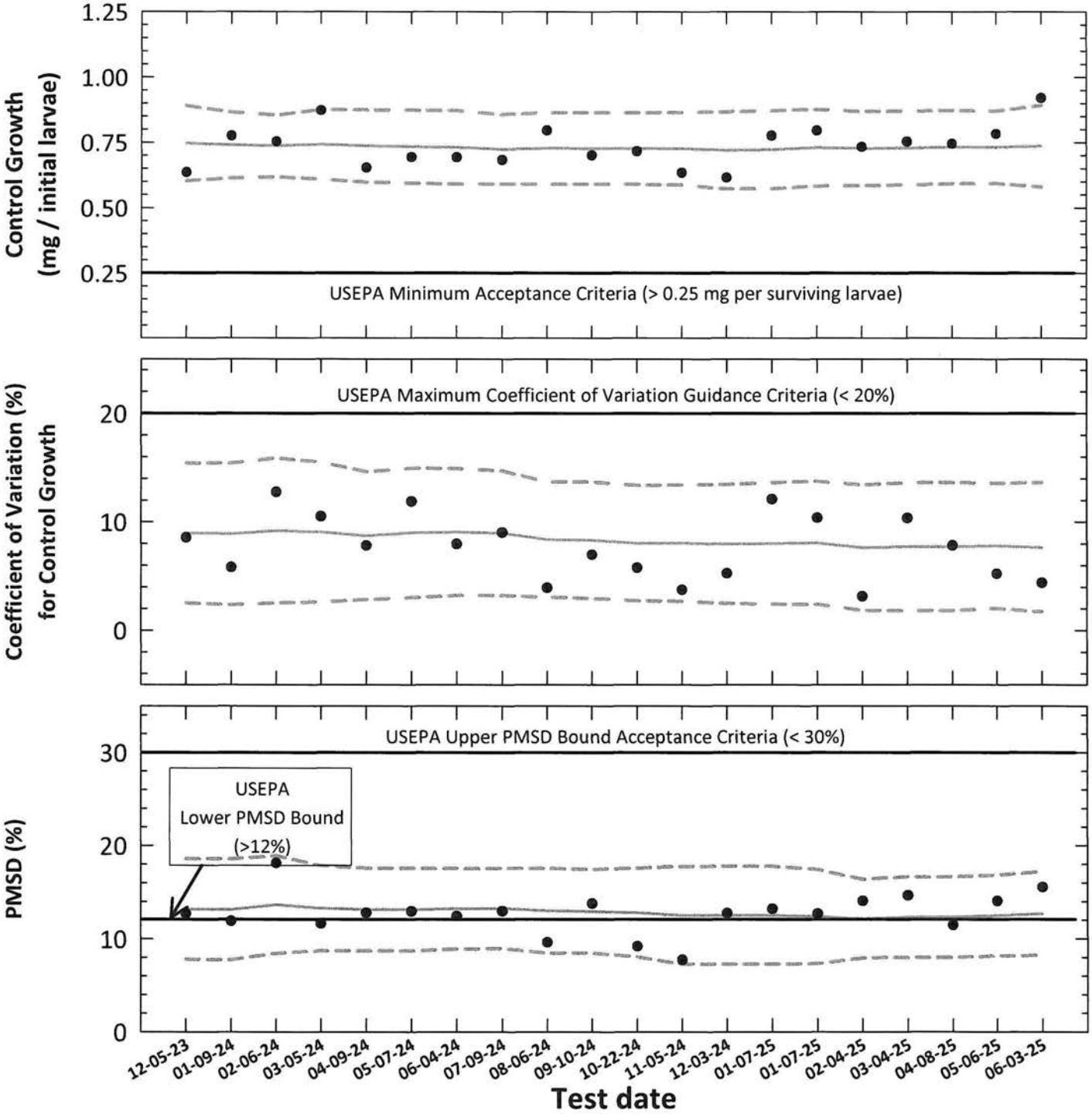
**$S_{A.75}$** = Standard deviation corresponding to the 75th percentile of CVs reported nationally by USEPA ($S_{A.75}$ = 0.38).

**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 | 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 |
| 2 | 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 |
| 3 | 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 |
| 4 | 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 |
| 5 | 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 |
| 6 | 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 |
| 7 | 06-04-24 | 100 | 0.693 | 8.0 | 0.0862 | 12.4 | 0.732 | 0.591 | 0.872 | 9.1 | 3.2 | 14.9 | 13.2 | 8.9 | 17.5 |
| 8 | 07-09-24 | 100 | 0.683 | 9.0 | 0.0883 | 12.9 | 0.724 | 0.591 | 0.857 | 9.0 | 3.2 | 14.7 | 13.2 | 8.9 | 17.5 |
| 9 | 08-06-24 | 100 | 0.796 | 3.9 | 0.0766 | 9.6 | 0.728 | 0.591 | 0.865 | 8.4 | 3.0 | 13.7 | 13.0 | 8.4 | 17.5 |
| 10 | 09-10-24 | 100 | 0.701 | 7.0 | 0.0964 | 13.8 | 0.728 | 0.591 | 0.865 | 8.3 | 2.9 | 13.7 | 12.9 | 8.5 | 17.4 |
| 11 | 10-22-24 | 100 | 0.717 | 5.8 | 0.0659 | 9.2 | 0.727 | 0.590 | 0.864 | 8.1 | 2.7 | 13.4 | 12.8 | 8.0 | 17.6 |
| 12 | 11-05-24 | 100 | 0.635 | 3.7 | 0.0491 | 7.7 | 0.727 | 0.588 | 0.866 | 8.0 | 2.7 | 13.4 | 12.5 | 7.3 | 17.8 |
| 13 | 12-03-24 | 100 | 0.615 | 5.3 | 0.0785 | 12.8 | 0.721 | 0.573 | 0.868 | 8.0 | 2.5 | 13.5 | 12.5 | 7.3 | 17.8 |
| 14 | 01-07-25 | 100 | 0.776 | 12.1 | 0.1024 | 13.2 | 0.723 | 0.573 | 0.872 | 8.0 | 2.4 | 13.7 | 12.5 | 7.3 | 17.8 |
| 15 | 01-07-25 | 100 | 0.796 | 10.4 | 0.1010 | 12.7 | 0.731 | 0.585 | 0.877 | 8.1 | 2.4 | 13.8 | 12.4 | 7.3 | 17.4 |
| 16 | 02-04-25 | 100 | 0.733 | 3.1 | 0.1030 | 14.1 | 0.727 | 0.585 | 0.869 | 7.6 | 1.8 | 13.4 | 12.2 | 7.9 | 16.4 |
| 17 | 03-04-25 | 100 | 0.754 | 10.4 | 0.1103 | 14.6 | 0.729 | 0.588 | 0.871 | 7.7 | 1.8 | 13.6 | 12.3 | 8.0 | 16.7 |
| 18 | 04-08-25 | 100 | 0.746 | 7.8 | 0.0855 | 11.5 | 0.733 | 0.593 | 0.873 | 7.7 | 1.8 | 13.7 | 12.3 | 8.0 | 16.7 |
| 19 | 05-06-25 | 100 | 0.783 | 5.2 | 0.1099 | 14.0 | 0.732 | 0.593 | 0.871 | 7.8 | 2.0 | 13.6 | 12.5 | 8.1 | 16.8 |
| 20 | 06-03-25 | 100 | 0.920 | 4.4 | 0.1429 | 15.5 | 0.736 | 0.580 | 0.893 | 7.7 | 1.7 | 13.6 | 12.7 | 8.2 | 17.2 |

*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 Sagmiser

Environmental Testing Solutions, Inc.

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

PpKClCR Test Number: **129**

| ***Dilution preparation information:*** | | | | | | | ***Comments:*** |
|---|---|---|---|---|---|---|---|
| KCl Stock INSS number: | | | INSS 7376 | | | | |
| 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: | 05-26-25 | *Artemia* CHM number: | CHM1385 |
| Hatch dates and times: | 06-02-25 1440 TO 06-03-25 0500 | ***Drying information for weight determination:*** | |
| Transfer vessel information: | pH = 8.04 S.U.<br>Temperature = 24.0 °C | Date / Time in oven: | 06-10-25 0620 |
| | | *Initial oven temperature: | 60 °C |
| Average transfer volume: | < 0.25 mL | Date / Time out of oven: | 06-11-25 0620 |
| | | *Final oven temperature: | 60 °C |
| | | Total drying time: | 24-HOURS |

*60°C Oven, Thermometer SN: 14-985B5

***Daily feeding and renewal information:***

| Day | Date | Morning feeding | | Afternoon feeding | | Test initiation, renewal, or termination | | MHSW batch used |
|---|---|---|---|---|---|---|---|---|
| | | Time | Analyst | Time | Analyst | Time | Analyst | |
| 0 | 06-03-25 | 0505 | JH | 1345 | JH | 0715 | JH | 05-28-25 A |
| 1 | 06-04-25 | 0500 | JH | 1230 | JH | 0710 | JH | ↓ |
| 2 | 06-05-25 | 0500 | JH | 1340 | JH | 0715 | JH | 05-28-25 E JH |
| 3 | 06-06-25 | 0600 | JH | 1200 | JH | 0800 | JH | ↓ |
| 4 | 06-07-25 | 0600 | JH | 1200 | JH | 0800 | JH | 06-02-25 A |
| 5 | 06-08-25 | 0600 | JH | 1130 | JH | 0800 | JH | ↓ |
| 6 | 06-09-25 | 0500 | JH | 1100 | JH | 0708 | JH | ↓ |
| 7 | 06-10-25 | | | | | 0617 | JH | |

***Chemical analyses:***

| Parameter | Reporting Limit | Method number | Meter | Serial number |
|---|---|---|---|---|
| pH | 0.1 S.U. | SM 4500-H+ B-2021 | Accumet AR20 | 93312452 |
| Dissolved Oxygen (D.O.) | 1.0 mg/L | SM 4500-O G-2021 | YSI Model 52CE | 08A100271 |
| Conductivity | 14.9 µmhos/cm | SM 2510 B-2021 | Accumet AR20 | 93312452 |
| Alkalinity | 5.0 mg $CaCO_3$/L | SM 2320 B-2021 | Accumet AR20 | 93312452 |
| Hardness | 5.0 mg $CaCO_3$/L | SM 2340 C-2021 | Not applicable | Not applicable |
| Temperature | 0.1°C | SM 2550B-2010 | Digital Thermometer | 130664685 |

| ***Control information:*** | | Acceptance criteria | ***Summary of test endpoints:*** | |
|---|---|---|---|---|
| % Mortality: | 0% | ≤ 20% | **7-day $LC_{50}$** (mg/L KCl) | 777.5 |
| Average weight per initial larvae: | 0.920 | | **NOEC** (mg/L KCl) | 600 |
| Average weight per surviving larvae: | 0.920 | ≥ 0.25 mg/larvae | **LOEC** (mg/L KCl) | 750 |
| | | | **ChV** (mg/L KCl) | 670.8 |
| | | | **$IC_{25}$** (mg/L KCl) | 676.8 |

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **129**

***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)<br>Tray color code: Lavender<br>Analyst: XL<br>Date: 05-22-25 | 15.47 | 11.37 | 12.69 | 11.86 | 13.45 | 13.36 | 14.30 | 14.58 | 12.79 | 14.47 | 13.68 | 14.56 |
| *B = Pan + Larvae weight (mg)<br>Analyst: JL<br>Date: 06-12-25 | 24.27 | 20.33 | 22.39 | 21.21 | 23.92 | 23.05 | 25.26 | 23.71 | 21.72 | 23.62 | 23.76 | 26.23 |
| C = Larvae weight (mg) = B – A<br>Analyst: JP | 8.80 | 8.96 | 9.70 | 9.35 | 10.47 | 9.69 | 10.96 | 9.13 | 8.93 | 9.15 | 10.08 | 11.67 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JP | 0.880 | 0.896 | 0.970 | 0.935 | 1.047 | 0.969 | 1.096 | 0.913 | 0.893 | 0.915 | 1.008 | 1.167 |
| Average weight per initial number of larvae (mg) / Percent reduction from control (%) | 0.920 | | | | 1.006 | | -9.3% | | 0.996 | | -8.2% | |

*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:*** |
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Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **129**

***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 | 8 2d | 8 2d | 10 | 9 1d | 5 3d | 4 6d | 5 3d | 5 3d |
| 2 | 10 | 10 | 10 | 10 | 8 | 8 | 10 | 9 | 4 1d | 4 | 3 2d | 4 1d |
| 3 | 10 | 10 | 10 | 10 | 7 1d | 8 | 10 | 7 2d | 4 | 4 | 1 2d | 4 |
| 4 | 10 | 10 | 10 | 10 | 7 | 8 | 7 3d | 7 | 4 | 4 | 1 | 4 |
| 5 | 10 | 10 | 10 | 10 | 6 1d | 6 2d | 6 1d | 6 1d | 3 1d | 4 | 1 | 4 |
| 6 | 10 | 10 | 9 1d | 10 | 6 | 5 1d | 6 | 5 1d | 3 | 4 | 1 | 4 |
| 7 | 10 | 10 | 9 | 9 1d | 6 | 5 | 6 | 5 | 3 | 3 1d | 1 | 3 1d |
| *A = Pan weight (mg)<br>Tray color code: Lavender<br>Analyst: XL<br>Date: 05-22-25 | 13.73 | 12.49 | 12.00 | 12.89 | 13.14 | 12.81 | 14.02 | 14.07 | 12.69 | 13.50 | 12.66 | 13.66 |
| *B = Pan + Larvae weight (mg)<br>Analyst: JL<br>Date: 06-12-25 | 24.09 | 22.48 | 21.60 | 21.75 | 19.13 | 17.28 | 19.32 | 18.40 | 15.92 | 15.66 | 13.69 | 16.14 |
| C = Larvae weight (mg) = B – A<br>Analyst: JP | 10.36 | 9.99 | 9.60 | 8.86 | 5.99 | 4.47 | 5.30 | 4.33 | 3.23 | 2.16 | 1.03 | 2.48 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: JP | 1.036 | 0.999 | 0.960 | 0.886 | 0.599 | 0.447 | 0.530 | 0.433 | 0.323 | 0.216 | 0.103 | 0.248 |
| Average weight per initial number of larvae (mg) | 0.970 | | | | 0.502 | | | | 0.223 | | | |
| Percent reduction from control (%) | | | -5.4% | | | | 45.4% | | | | 75.8% | |

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

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

***Comments:***

Environmental Testing Solutions, Inc.

**Species: *Pimephales promelas*** PpKClCR Test Number: **129**

***Survival and Growth Data***

| Day | 1050 mg KCl/L | | | |
|---|---|---|---|---|
| | Y | Z | AA | BB |
| 0 | 10 | 10 | 10 | 10 |
| 1 | 4 6d | 3 7d | 4 6d | 3 7d |
| 2 | 1 3d | 1 2d | 1 3d | 2 1d |
| 3 | 1 | 0 1d | 1 | 2 |
| 4 | 1 | 0 | 1 | 2 |
| 5 | 1 | 0 | 0 1d | 0 2d |
| 6 | 0 1d | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 |
| *A = Pan weight (mg)<br>Tray color code: Lavender<br>Analyst: XL<br>Date: 05-22-25 | 14.27 | 13.62 | 13.99 | 14.40 |
| *B = Pan + Larvae weight (mg)<br>Analyst:<br>Date: | | | | |
| C = Larvae weight (mg) = B – A<br>Analyst: | | | | M 06-10-25 |
| Weight per initial number of larvae (mg) = C / Initial number of larvae<br>Analyst: yl | 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:*** |
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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: 129

Test dates: June 03-10, 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 | 15.47 | 24.27 | 8.80 | 0.880 | 0.920 | 4.4 | 0.880 | 100.0 | 0.920 | 4.4 | Not applicable |
| | B | 10 | 10 | 11.37 | 20.33 | 8.96 | 0.896 | | | 0.896 | | | | |
| | C | 10 | 10 | 12.69 | 22.39 | 9.70 | 0.970 | | | 0.970 | | | | |
| | D | 10 | 10 | 11.86 | 21.21 | 9.35 | 0.935 | | | 0.935 | | | | |
| 300 | E | 10 | 10 | 13.45 | 23.92 | 10.47 | 1.047 | 1.006 | 8.1 | 1.047 | 100.0 | 1.006 | 8.1 | -9.3 |
| | F | 10 | 10 | 13.36 | 23.05 | 9.69 | 0.969 | | | 0.969 | | | | |
| | G | 10 | 10 | 14.30 | 25.26 | 10.96 | 1.096 | | | 1.096 | | | | |
| | H | 10 | 10 | 14.58 | 23.71 | 9.13 | 0.913 | | | 0.913 | | | | |
| 450 | I | 10 | 10 | 12.79 | 21.72 | 8.93 | 0.893 | 0.996 | 12.5 | 0.893 | 100.0 | 0.996 | 12.5 | -8.2 |
| | J | 10 | 10 | 14.47 | 23.62 | 9.15 | 0.915 | | | 0.915 | | | | |
| | K | 10 | 10 | 13.68 | 23.76 | 10.08 | 1.008 | | | 1.008 | | | | |
| | L | 10 | 10 | 14.56 | 26.23 | 11.67 | 1.167 | | | 1.167 | | | | |
| 600 | M | 10 | 10 | 13.73 | 24.09 | 10.36 | 1.036 | 1.022 | 3.6 | 1.036 | 95.0 | 0.970 | 6.6 | -5.4 |
| | N | 10 | 10 | 12.49 | 22.48 | 9.99 | 0.999 | | | 0.999 | | | | |
| | O | 10 | 9 | 12.00 | 21.60 | 9.60 | 1.067 | | | 0.960 | | | | |
| | P | 10 | 9 | 12.89 | 21.75 | 8.86 | 0.984 | | | 0.886 | | | | |
| 750 | Q | 10 | 6 | 13.14 | 19.13 | 5.99 | 0.998 | 0.910 | 6.6 | 0.599 | 55.0 | 0.502 | 15.4 | 45.4 |
| | R | 10 | 5 | 12.81 | 17.28 | 4.47 | 0.894 | | | 0.447 | | | | |
| | S | 10 | 6 | 14.02 | 19.32 | 5.30 | 0.883 | | | 0.530 | | | | |
| | T | 10 | 5 | 14.07 | 18.40 | 4.33 | 0.866 | | | 0.433 | | | | |
| 900 | U | 10 | 3 | 12.69 | 15.92 | 3.23 | 1.077 | 0.913 | 18.4 | 0.323 | 25.0 | 0.223 | 41.1 | 75.8 |
| | V | 10 | 3 | 13.50 | 15.66 | 2.16 | 0.720 | | | 0.216 | | | | |
| | W | 10 | 1 | 12.66 | 13.69 | 1.03 | 1.030 | | | 0.103 | | | | |
| | X | 10 | 3 | 13.66 | 16.14 | 2.48 | 0.827 | | | 0.248 | | | | |
| 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: 0.1352

PMSD: 14.7

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.

ETS Environmental Testing Solutions, Inc.

# Statistical Analyses

Entered and Reviewed by Jim Sumner

## Larval Fish Growth and Survival Test-7 Day Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 6/3/2025 | Test ID: | PpKClCR | Sample ID: | REF-Ref Toxicant |
| End Date: | 6/10/2025 | 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 | 1.0000 | 0.9000 | 0.9000 |
| 750 | 0.6000 | 0.5000 | 0.6000 | 0.5000 |
| 900 | 0.3000 | 0.3000 | 0.1000 | 0.3000 |
| 1050 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | | | 0 | 40 |
| 300 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | 18.00 | 10.00 | 0 | 40 |
| 450 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 4 | 18.00 | 10.00 | 0 | 40 |
| 600 | 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 | 0.2500 | 0.2500 | 0.5152 | 0.3218 | 0.5796 | 25.030 | 4 | 10.00 | 10.00 | 30 | 40 |
| 1050 | 0.0000 | 0.0000 | 0.1588 | 0.1588 | 0.1588 | 0.000 | 4 | | | 40 | 40 |

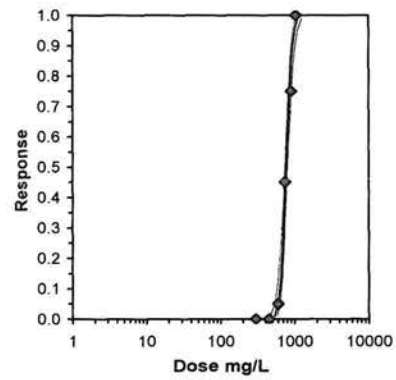
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01) | 0.84536 | 0.884 | -1.321 | 3.15392 |
| Equality of variance cannot be confirmed | | | | |

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

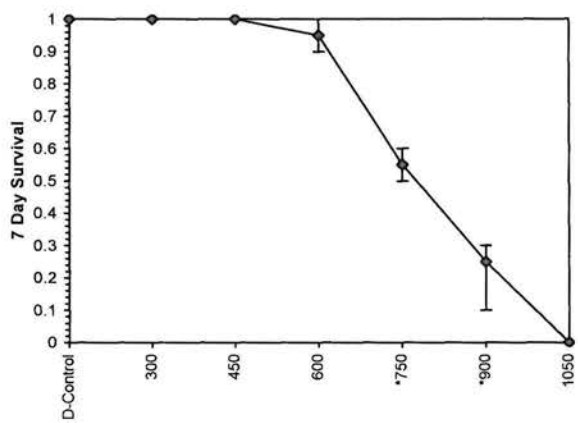
### Maximum Likelihood-Probit

| Parameter | Value | SE | 95% Fiducial Limits | | Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Slope | 14.5583 | 1.81341 | 11.0041 | 18.1126 | 0 | 2.90233 | 9.48773 | 0.5743 | 2.89069 | 0.06869 | 4 |
| Intercept | -37.084 | 5.26025 | -47.394 | -26.773 | | | | | | | |
| TSCR | | | | | | | | | | | |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2.674 | 538.135 | 470.683 | 585.645 |
| EC05 | 3.355 | 599.381 | 541.31 | 640.432 |
| EC10 | 3.718 | 634.831 | 582.698 | 672.278 |
| EC15 | 3.964 | 659.925 | 612.044 | 695.053 |
| EC20 | 4.158 | 680.575 | 636.113 | 714.046 |
| EC25 | 4.326 | 698.806 | 657.215 | 731.086 |
| EC40 | 4.747 | 746.938 | 711.599 | 777.971 |
| EC50 | 5.000 | 777.475 | 744.485 | 809.754 |
| EC60 | 5.253 | 809.261 | 776.948 | 844.943 |
| EC75 | 5.674 | 865.001 | 829.601 | 911.745 |
| EC80 | 5.842 | 888.172 | 850.193 | 941.11 |
| EC85 | 6.036 | 915.964 | 874.16 | 977.296 |
| EC90 | 6.282 | 952.172 | 904.469 | 1025.73 |
| EC95 | 6.645 | 1008.49 | 950.148 | 1103.34 |
| EC99 | 7.326 | 1123.26 | 1039.79 | 1267.98 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

# Statistical Analyses

Entered and Reviewed by Jim Summer

## Larval Fish Growth and Survival Test-7 Day Growth

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 6/3/2025 | Test ID: | PpKClCR | Sample ID: | REF-Ref Toxicant |
| End Date: | 6/10/2025 | 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 | 0.8800 | 0.8960 | 0.9700 | 0.9350 |
| 300 | 1.0470 | 0.9690 | 1.0960 | 0.9130 |
| 450 | 0.8930 | 0.9150 | 1.0080 | 1.1670 |
| 600 | 1.0360 | 0.9990 | 0.9600 | 0.8860 |
| 750 | 0.5990 | 0.4470 | 0.5300 | 0.4330 |
| 900 | 0.3230 | 0.2160 | 0.1030 | 0.2480 |
| 1050 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

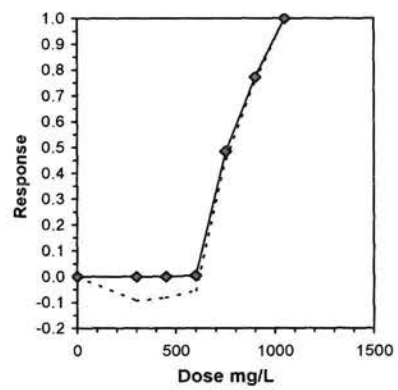
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| D-Control | 0.9203 | 1.0000 | 0.9203 | 0.8800 | 0.9700 | 4.392 | 4 | | | | 0.9741 | 1.0000 |
| 300 | 1.0063 | 1.0935 | 1.0063 | 0.9130 | 1.0960 | 8.073 | 4 | -1.457 | 2.290 | 0.1352 | 0.9741 | 1.0000 |
| 450 | 0.9958 | 1.0820 | 0.9958 | 0.8930 | 1.1670 | 12.510 | 4 | -1.279 | 2.290 | 0.1352 | 0.9741 | 1.0000 |
| 600 | 0.9703 | 1.0543 | 0.9703 | 0.8860 | 1.0360 | 6.614 | 4 | -0.847 | 2.290 | 0.1352 | 0.9703 | 0.9961 |
| 750 | 0.5023 | 0.5458 | 0.5023 | 0.4330 | 0.5990 | 15.413 | 4 | | | | 0.5023 | 0.5156 |
| 900 | 0.2225 | 0.2418 | 0.2225 | 0.1030 | 0.3230 | 41.088 | 4 | | | | 0.2225 | 0.2284 |
| 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) | 0.9577 | 0.844 | 0.56696 | 0.26196 |
| Bartlett's Test indicates equal variances (p = 0.36) | 3.23952 | 11.3449 | | |

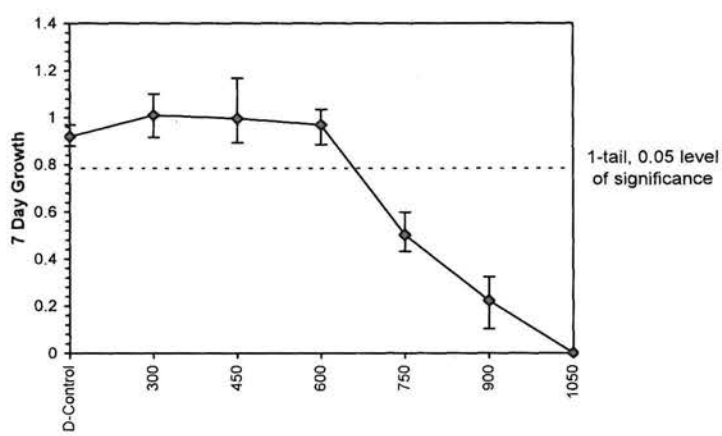
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 600 | >600 | | | 0.13516 | 0.14687 | 0.00588 | 0.00697 | 0.49553 | 3, 12 |
| Treatments vs D-Control | | | | | | | | | | |

### Linear Interpolation (200 Resamples)

| Point | mg/L | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 614.38 | 24.90 | 498.31 | 618.15 | -4.5012 |
| IC10 | 629.99 | 7.86 | 597.27 | 635.56 | -2.8831 |
| IC15 | 645.60 | 6.73 | 616.17 | 655.07 | -1.0445 |
| IC20 | 661.21 | 6.73 | 634.29 | 674.38 | -0.4552 |
| IC25 | 676.82 | 7.07 | 651.80 | 693.61 | 0.1105 |
| IC40 | 723.65 | 9.61 | 697.73 | 755.21 | 0.8469 |
| IC50 | 758.15 | 14.56 | 722.22 | 807.85 | 0.7440 |




### Dose-Response Plot



Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **129**

## *Daily Chemistry:*

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

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | |
| | Analyst | XL | XL | XL | XL | XL | XL |
| Concentration | Parameter | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.74 | 7.78 | 7.75 | 7.72 | 7.79 | 7.54 |
| | Dissolved oxygen (mg/L) | 8.6 | 8.0 | 8.4 | 7.8 | 8.4 | 7.2 |
| | Conductivity (µmhos/cm) | 294 | | 310 | | 313 | |
| | Alkalinity (mg $CaCO_3$/L) | 62 | | | | 59 | |
| | Hardness (mg $CaCO_3$/L) | 88 | | | | 90 | |
| | Temperature (°C) | 24.8 | 24.8 | 24.7 | 24.7 | 24.7 | 24.5 |
| 300 mg KCl/L | pH (S.U.) | 7.89 | 7.75 | 7.87 | 7.67 | 7.92 | 7.53 |
| | Dissolved oxygen (mg/L) | 8.5 | 7.9 | 8.3 | 7.7 | 8.4 | 7.1 |
| | Conductivity (µmhos/cm) | 832 | | 848 | | 861 | |
| | Temperature (°C) | 24.9 | 24.7 | 24.6 | 24.4 | 24.7 | 24.2 |
| 450 mg KCl/L | pH (S.U.) | 7.88 | 7.76 | 7.89 | 7.68 | 7.92 | 7.52 |
| | Dissolved oxygen (mg/L) | 8.5 | 7.9 | 8.3 | 7.8 | 8.4 | 6.9 |
| | Conductivity (µmhos/cm) | 1070 | | 1130 | | 1120 | |
| | Temperature (°C) | 24.9 | 24.7 | 24.6 | 24.4 | 24.6 | 24.4 |
| 600 mg KCl/L | pH (S.U.) | 7.89 | 7.76 | 7.89 | 7.70 | 7.92 | 7.53 |
| | Dissolved oxygen (mg/L) | 8.5 | 7.9 | 8.3 | 7.8 | 8.4 | 7.1 |
| | Conductivity (µmhos/cm) | 1320 | | 1380 | | 1370 | |
| | Temperature (°C) | 25.0 | 24.7 | 24.7 | 24.6 | 24.6 | 24.5 |
| 750 mg KCl/L | pH (S.U.) | 7.89 | 7.77 | 7.89 | 7.69 | 7.92 | 7.54 |
| | Dissolved oxygen (mg/L) | 8.5 | 7.9 | 8.3 | 7.7 | 8.4 | 7.0 |
| | Conductivity (µmhos/cm) | 1570 | | 1640 | | 1640 | |
| | Temperature (°C) | 24.8 | 24.6 | 24.7 | 24.9 | 24.6 | 24.5 |
| 900 mg KCl/L | pH (S.U.) | 7.89 | 7.78 | 7.89 | 7.69 | 7.92 | 7.54 |
| | Dissolved oxygen (mg/L) | 8.5 | 7.9 | 8.3 | 7.7 | 8.4 | 7.0 |
| | Conductivity (µmhos/cm) | 1830 | | 1900 | | 1890 | |
| | Temperature (°C) | 24.8 | 24.6 | 24.7 | 24.6 | 24.8 | 24.6 |
| 1050 mg KCl/L | pH (S.U.) | 7.89 | 7.78 | 7.90 | 7.75 | 7.93 | 7.60 |
| | Dissolved oxygen (mg/L) | 8.5 | 7.9 | 8.3 | 8.0 | 8.4 | 7.4 |
| | Conductivity (µmhos/cm) | 2110 | | 2190 | | 2170 | |
| | Temperature (°C) | 24.8 | 24.6 | 24.7 | 24.6 | 24.6 | 24.6 |
| | | Initial | Final | Initial | Final | Initial | Final |

Environmental Testing Solutions, Inc.

**Species:** ***Pimephales promelas*** PpKClCR Test Number: **129**

| | | Day (Analyst identified for each day, performed pH, D.O. and conductivity measurements only.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | Analyst | XL | BSL | BSL | BSL | BSL | XL | XL | [illegible] |
| Concentration | Parameter | | | | | | | | |
| CONTROL, MHSW | pH (S.U.) | 7.80 | 7.68 | 7.88 | 7.58 | 7.87 | 7.60 | 7.81 | 7.49 |
| | Dissolved oxygen (mg/L) | 8.4 | 7.6 | 8.3 | 7.1 | 8.4 | 7.4 | 8.3 | 5.8 |
| | Conductivity (µmhos/cm) | 298 | | 299 | | 295 | | 305 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 60 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | 0640-25 A | 90 | | | | | 0640-2 A |
| | Temperature (°C) | 24.6 | 24.5 | 24.7 | 24.4 | 24.6 | 24.8 | 24.7 | 24.3 |
| 300 mg KCl/L | pH (S.U.) | 7.89 | 7.68 | 7.92 | 7.58 | 7.94 | 7.57 | 7.92 | 7.39 |
| | Dissolved oxygen (mg/L) | 8.4 | 7.4 | 8.4 | 6.9 | 8.4 | 7.2 | 8.3 | 5.8 |
| | Conductivity (µmhos/cm) | 816 | | 814 | | 818 | | 850 | |
| | Temperature (°C) | 24.6 | 24.6 | 24.6 | 24.6 | 24.6 | 24.6 | 24.6 | 24.4 |
| 450 mg KCl/L | pH (S.U.) | 7.90 | 7.71 | 7.97 | 7.58 | 8.01 | 7.58 | 7.92 | 7.42 |
| | Dissolved oxygen (mg/L) | 8.4 | 7.4 | 8.4 | 6.9 | 8.4 | 7.2 | 8.3 | 5.8 |
| | Conductivity (µmhos/cm) | 1070 | | 1070 | | 1080 | | 1090 | |
| | Temperature (°C) | 24.7 | 24.3 | 24.6 | 24.3 | 24.6 | 24.6 | 24.6 | 24.4 |
| 600 mg KCl/L | pH (S.U.) | 7.91 | 7.71 | 7.98 | 7.58 | 8.01 | 7.59 | 7.94 | 7.40 |
| | Dissolved oxygen (mg/L) | 8.4 | 7.5 | 8.4 | 6.7 | 8.4 | 7.2 | 8.3 | 5.4 |
| | Conductivity (µmhos/cm) | 1330 | | 1330 | | 1300 | | 1370 | |
| | Temperature (°C) | 24.6 | 24.6 | 24.8 | 24.5 | 24.6 | 24.9 | 24.8 | 24.6 |
| 750 mg KCl/L | pH (S.U.) | 7.91 | 7.73 | 8.01 | 7.59 | 8.02 | 7.58 | 7.94 | 7.40 |
| | Dissolved oxygen (mg/L) | 8.4 | 7.5 | 8.4 | 6.8 | 8.4 | 7.1 | 8.3 | 5.2 |
| | Conductivity (µmhos/cm) | 1560 | | 1580 | | 1570 | | 1590 | |
| | Temperature (°C) | 24.7 | 24.6 | 24.8 | 24.5 | 24.5 | 24.5 | 24.8 | 24.7 |
| 900 mg KCl/L | pH (S.U.) | 7.92 | 7.74 | 8.01 | 7.63 | 8.02 | 7.61 | 7.94 | 7.39 |
| | Dissolved oxygen (mg/L) | 8.4 | 7.4 | 8.4 | 6.8 | 8.4 | 7.2 | 8.3 | 5.5 |
| | Conductivity (µmhos/cm) | 1820 | | 1840 | | 1840 | | 1860 | |
| | Temperature (°C) | 24.7 | 24.6 | 24.6 | 24.7 | 24.5 | 24.5 | 24.7 | 24.5 |
| 1050 mg KCl/L | pH (S.U.) | 7.92 | 7.81 | 8.01 | 7.69 | 8.02 | 7.63 | 7.95 | |
| | Dissolved oxygen (mg/L) | 8.4 | 7.8 | 8.4 | 7.3 | 8.4 | 7.3 | 8.3 | |
| | Conductivity (µmhos/cm) | 2080 | | 2090 | | 2090 | | 2140 | |
| | Temperature (°C) | 24.7 | 24.5 | 24.6 | 24.4 | 24.6 | 24.5 | 24.7 | 24.5 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |