

## Biomonitoring Report

prepared for  
**WirxGroup LLC**

### **CleanWirx Run-off from Treated Plate Metal.**

INLAND SILVERSIDE (*Menidia beryllina*) LARVAL SURVIVAL & GROWTH TEST, EPA-821-R-02-014: METHOD 1006

This test was initiated September 23, 2013 at 1423

#### ***M. beryllina*; EE USA Project No.: Q-625-13**

SURVIVAL NOEC/LOEC = 25.0%/35.0% LS

GROWTH NOEC/LOEC = <12.0%/12.0% LS

LPC % CV = 6.4

MYSID (*Mysidopsis bahia*) SURVIVAL, GROWTH, AND FECUNDITY TEST, EPA-821-R-02-014: METHOD 1007

This test was initiated September 23, 2013 at 1422

#### ***M. bahia*; EE USA Project No.: Q-626-13**

SURVIVAL NOEC/LOEC = 1.1%/1.8% LS

GROWTH NOEC/LOEC = 0.6%/1.1% LS

LPC% CV = 14.9

Report Date: October 11, 2013

by

#### **ENVIRONMENTAL ENTERPRISES USA, INC.**

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*This report narrative contains seven pages. The results and conclusions presented in this report apply only to the sample(s) tested. All results included in this report are from a valid test.*

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**INLAND SILVERSIDE (*Menidia beryllina*) LARVAL SURVIVAL & GROWTH TEST**  
**EPA-821-R-02-014: METHOD 1006**

**TEST OVERVIEW**

A 7-day static-renewal toxicity test was conducted by **Environmental Enterprises USA, Inc. (EE USA)** to determine toxicity of the lab sample (LS) CleanWirx, after being used to treat plate metal, to *Menidia beryllina* larvae. Methods, materials, and results are presented in this document. Test organisms were cultured at EE USA and were 11-days-old when this test was initiated. Synthetic seawater was used as the performance control solution and diluent in this test. Five replicates of the performance control solution and five LS concentrations were prepared initially and renewed daily. LS concentrations tested were 12.0, 17.0, 25.0, 35.0, and 50.0%. Test concentrations tested were determined from a range finding test that was initiated August 29, 2013. This test was initiated September 23, 2013, at 1423 and completed September 30, 2013, at 0927.

**MATERIALS AND METHODS**

Materials and methods for the work performed are stated in EPA-821-R-02-014: Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Actual materials and methods are detailed below. This test was performed with strict adherence to the requirements of Method 1006 and/or the Western Gulf of Mexico OCS General Permit. The recommendations and suggestions made elsewhere in EPA-821-R-02-014 were incorporated whenever applicable to optimize the experimental design. Dilution water was prepared with hw-MARINEMIX + Bio-elements and Crystal Sea Marinemix Bioassay Laboratory Formula sea salts (80:20) and deionized water and adjusted to 25 parts per thousand (ppt) salinity.

*M. beryllina* was cultured and maintained at 24±1°C and 25 ppt salinity. Several clutches from different females comprised the embryo pool from which test organism population hatched. Test organisms were fed 250 – 500 µl of a standardized suspension of less than 24-hour-old *Artemia* nauplii twice daily by replicate. The standard suspension is equal to 0.05 g wet weight strained nauplii per ml synthetic seawater. Test organisms were not fed on Day 7. One day prior to test initiation, eight inland silverside minnows were transferred randomly into 30 test chambers with 250 ml synthetic seawater. These test chambers were then placed in the environmental chamber.

Sensitivity of test organisms to a known toxicant was determined by performing a chronic Standard Reference Toxicant (SRT) test, MN1310, with potassium chloride (Sigma Chemical, Lot SLBC2414V). The SRT test was initiated on September 4, 2013, with 11-day-old *M. beryllina* larvae

	SURVIVAL	GROWTH
NOEC:	980 mg/l	980 mg/l
LOEC:	1400 mg/l	>980 mg/l

The sample used in this test was delivered to **EE USA** on September 20, 2013. This sample was stored at 0.1 to 6°C and used to prepare the initial and subsequent renewal test solutions. Test chambers were labeled with replicate identification, and **EE USA's** project number. Six treatments, five LS concentrations and a laboratory performance control were prepared and pH was measured in the undiluted LS sample daily.

Each treatment was poured into a new acid-washed 1-gallon plastic container and placed in an environmental chamber to warm up to test temperature. After the test solutions reached test temperature, initial water quality parameters (temperature, dissolved oxygen (DO), and salinity) were measured. At the end of each 24-hour exposure period, prior to renewal, the ending DO, temperature, salinity, and pH in each treatment were recorded. Alkalinity, pH, and salinity were measured in the laboratory performance control September 23, September 25, and September 27, 2013.

On Day 0, the preloaded replicate test chambers were removed from the environmental chamber and carefully examined. Dead or injured larvae were replaced with organisms from the same batch and this test was initiated by renewal: 90% of the treatment solution, excess food, and debris were poured or siphoned out of each replicate. Aliquots of freshly prepared treatments were poured gently into each replicate as appropriate and then this test was placed in the environmental chamber. Surviving test organisms were disturbed as little as possible during renewal. On Days 1-6, the test was renewed.

Every 24 hours, survival was recorded. After seven days, the final survival data were recorded and this test was terminated. Surviving *M. beryllina* were rinsed in deionized water, placed on a tared weighing dish, and dried at  $60 \pm 4^\circ\text{C}$  for 24 hours by replicate. After cooling for one hour, dried *M. beryllina* were weighed and the average individual dry weight for each replicate was calculated. The average individual dry weight is equal to the replicate weight divided by the number of original larvae. For evaluating test acceptability criteria, the mean dry weight and percent coefficient of variation (%CV) were calculated using the number of surviving *M. beryllina* in each replicate. The mean dry weight of surviving *M. beryllina* in the control was 1.148 mg and the highest %CV for lethal and sublethal effects for the control was 6.4%. The test acceptability criteria for mean dry weight for surviving *M. beryllina* in the control is  $\geq 0.50$  mg and the test acceptability criteria for %CV in the control and critical dilution for lethal and sublethal effects is  $\leq 40$ .

#### Summary of Experimental Conditions

Test Organisms:	11-day-old <i>Menidia beryllina</i> larvae
Dilution Water:	Synthetic seawater, 25 ppt salinity.
Temperature:	$25 \pm 1^\circ\text{C}$
Photoperiod:	16 hours light; 8 hours dark
Test Chambers:	Rectangular Pyrex dish, 21 cm x 11 cm x 7 cm. Total volume = 1.45 L
Test Solution Volume:	500 ml
Aeration:	Yes. On Day 1.
Test Solution Renewal:	Yes

### RESULTS AND CONCLUSION

The response used in statistical analysis of survival data was the proportion of surviving test organisms per replicate. These proportions were transformed by the Arc Sine Square Root Transformation and then tested for normal distribution and homogeneity of variance using Shapiro-Wilk's and Bartlett's tests, respectively. Survival data were normally distributed, unequal in variance, and evaluated by the nonparametric alternative, Steel's Many-One Rank test. The No Observed Effect Concentration (NOEC) for impaired *M. beryllina* survival was 25.00% LS. The Lowest Observed Effect Concentration (LOEC) was 35.0% LS. Dunnett's test was used to determine the minimum statistically significant percent difference (MSDp) between survival in the control and survival at any LS concentration tested. For this *M. beryllina* survival data set, the MSDp was 10.0%.

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The response used in growth data analysis was the average individual dry weight for each replicate: replicate weight divided by the number of original larvae. Growth data were not transformed and concentrations demonstrating significant mortality are routinely excluded from subsequent data analysis. Growth data were tested for normal distribution and homogeneity of variance using Shapiro Wilk's and Bartlett's tests, respectively. Growth data were normally distributed, equal in variance, and further evaluated by the parametric alternative, Dunnett's Test. The NOEC for impaired *M. beryllina* growth was <12.0% LS. The LOEC was 12.0% LS. Dunnett's test was used to determine the MSDp between growth in the control and growth at any PW concentration tested. For this *M. beryllina* growth data set, the MSDp was 15.5%.

Survival of *M. beryllina* larvae exposed to CleanWirx was reduced significantly at 35.0% LS (the LOEC). Growth was reduced significantly at 12.0% LS (the LOEC). Survival in the concurrent laboratory performance control was 100.0%.

**MYSID (*Mysidopsis bahia*) SURVIVAL, GROWTH, AND FECUNDITY TEST**  
**EPA-821-R-02-014: METHOD 1007**

**TEST OVERVIEW**

A 7-day static-renewal toxicity test was conducted by **EE USA** to determine toxicity of the lab sample (LS) CleanWirx, after being used to treat plate metal, to *Mysidopsis bahia* juveniles. Methods, materials, and results are presented in this document. Organisms used in this test were cultured at **EE USA** and 7-days-old when this test was initiated. Synthetic seawater was used as the performance control solution and diluent in this test. Eight replicates of the performance control solution and five LS concentrations were prepared initially and renewed daily. LS concentrations tested were 0.6, 1.1, 1.8, 3.0, and 5.0%. Test concentrations tested were determined from a range finding test that was initiated August 29 and September 3, 2013. This test was initiated September 23, 2013, at 1422 and completed September 30, 2013, at 1020.

**MATERIALS AND METHODS**

Materials and methods for the work performed are stated in EPA-821-R-02-014: Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Actual materials and methods are detailed below. This test was performed with strict adherence to the requirements of Method 1007 and/or the Western Gulf of Mexico OCS General Permit. The recommendations and suggestions made elsewhere in EPA-821-R-02-014 were incorporated whenever applicable to optimize the experimental design. Dilution water was prepared with hw-MARINEMIX + Bio-elements and Crystal Sea Marinemix Bioassay Laboratory Formula sea salts (80:20) and deionized water and adjusted to 25 parts per thousand (ppt) salinity.

*M. bahia* was cultured and maintained at  $24 \pm 1^\circ\text{C}$  and 25 ppt salinity. Six days before initiating this test, approximately 500, 12- to 24-hour-old mysids were collected from breeding cultures, moved to a holding system, and acclimated to  $26 \pm 1^\circ\text{C}$ . Test organisms were fed 150 – 250  $\mu\text{l}$  of a standardized suspension of less than 24-hour-old *Artemia* nauplii twice daily by replicate. The standard suspension is equal to 0.05 g wet weight strained nauplii per ml synthetic seawater.

Sensitivity of test organisms to a known toxicant was determined by performing a chronic Standard Reference Toxicant (SRT) test MB1310, with potassium chloride (Sigma Chemical, Lot SLBC2414V). The SRT test was initiated on September 4, 2013, with 7-day-old *M. bahia*.

	SURVIVAL	GROWTH
NOEC:	416 mg/l	416 mg/l
LOEC:	694 mg/l	>416 mg/l

The sample used in this test was delivered to EE USA on September 20, 2013. This sample was stored at 0.1 to  $6^\circ\text{C}$  and used to prepare the initial and subsequent renewal test solutions. Test chambers were labeled with replicate identification, and EE USA's project number. Six treatments, five LS concentrations and a laboratory performance control, were prepared and pH was measured in the undiluted LS sample daily.

Each treatment was poured into a new acid-washed 1-gallon plastic container and placed in an environmental chamber to warm up to test temperature. After the test solutions reached test temperature, initial water quality parameters (temperature, dissolved oxygen (DO), and salinity) were measured. At the end of each 24-hour exposure period, prior to renewal, the ending DO, temperature, salinity, and pH in each treatment were recorded. Alkalinity, pH, and salinity were measured in the laboratory performance control September 23, September 25, and September 27, 2013.

On Day 0, the treatments were poured into their respective test chambers, five *M. bahia* juveniles were distributed randomly to each, and then this test was placed in the environmental chamber. On Days 1-6, the test was renewed: 90% of the treatment solution, excess food, and debris were poured or siphoned out of each replicate. Aliquots of freshly prepared treatments were poured gently into each replicate as appropriate. Surviving test organisms were disturbed as little as possible during renewal.

Every 24 hours, survival was recorded. After seven days, the final survival data were recorded and this test was terminated. Surviving *M. bahia* were rinsed in deionized water, placed on a tared weighing dish, and dried at  $60 \pm 4^\circ\text{C}$  for 24 hours by replicate. After cooling for one hour, dried *M. bahia* were weighed and the average individual dry weight for each replicate was calculated. The average individual dry weight is equal to the replicate weight divided by the number of original mysids. For evaluating test acceptability criteria, the mean dry weight and percent coefficient of variation (%CV) were calculated using the number of surviving *M. bahia* in each replicate. The mean dry weight of surviving *M. bahia* in the control was 0.322 mg and the highest %CV for lethal and sublethal effects for the control was 14.9%. The test acceptability criteria for mean dry weight for surviving *M. bahia* in the control is  $\geq 0.20$  mg and the test acceptability criteria for %CV in the control and critical dilution for lethal and sublethal effects is  $\leq 40$ .

#### Summary of Experimental Conditions

Test Organisms:	7-day-old <i>Mysidopsis bahia</i> juveniles
Dilution Water:	Synthetic seawater, 25 ppt salinity.
Temperature:	$26 \pm 1^\circ\text{C}$
Photoperiod:	16 hours light; 8 hours dark
Test Chambers:	Disposable plastic cups, 9 cm in diameter. Total volume = 300 ml.
Test Solution Volume:	150 ml
Aeration:	Yes. On Day 1.
Test Solution Renewal:	Yes

### RESULTS AND CONCLUSION

The response used in statistical analysis of survival data was the proportion of surviving test organisms per replicate. These proportions were transformed by the Arc Sine Square Root Transformation and then tested for normal distribution and homogeneity of variance using Shapiro-Wilk's and Bartlett's tests, respectively. Survival data were not normally distributed and were further evaluated by the nonparametric alternative, Steel's Many-One Rank Test. The NOEC for impaired *M. bahia* survival was 1.1% LS. The LOEC was 1.8% LS. Dunnett's test was used to determine the MSDp between survival in the control and survival at any LS concentration tested. For this *M. bahia* survival data set, the MSDp was 13.4%.

The response used in growth data analysis was the average individual dry weight for each replicate: replicate weight divided by the number of original larvae. Growth data were not transformed and concentrations demonstrating significant mortality are routinely excluded from subsequent data analysis. Growth data were tested for normal distribution and homogeneity of variance using Shapiro Wilk's and Bartlett's tests, respectively. Growth data were not normally distributed, equal in variance, and further evaluated by the nonparametric alternative, Steel's Many-One Rank Test. The NOEC for impaired *M. bahia* growth was 0.6% LS. The LOEC was 1.1% LS. Dunnett's test was used to determine the MSDp between growth in the control and growth at any LS concentration tested. For this *M. bahia* growth data set, the MSDp was 14.6%.

Survival of *M. bahia* exposed to CleanWirx was reduced significantly at 1.8% LS (the LOEC). Growth was reduced significantly at 1.1% LS (the LOEC). Survival in the concurrent laboratory performance control was 100.0%.

## **REFERENCES**

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