

HOW DO YOU COMPLETE AN ACCEPTABLE TEST UNDER THE OSCPP GUIDELINES?

APPLICATION NOTE

## Sectioned PVC Pipe and Bent Stainless Steel Screen Substrates: A 96-Hour Static Acute Survivability Trial with the Freshwater Amphipod (*Gammarus Pseudolimnaeus*)

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### INTRODUCTION

The freshwater amphipod, *Gammarus pseudolimnaeus*, is frequently used to conduct acute toxicity tests. The survival of gammarids in control groups (>90%) is essential to complete an acceptable test under the OSCPP guidelines.<sup>1</sup> A preliminary review of historical range-finding and definitive control data (n=22) shows that 36% of tests had unacceptable control survival at test termination, and that repeat testing was frequently required.

Test chambers containing organisms traditionally have been 2L glass beakers filled with ~500-1500 mL of solution. However, considerable evidence shows that aquatic amphipods thrive in and around substrates in their environment, both in brackish<sup>2</sup> and freshwater<sup>3</sup>, and specifically prefer substrates that afford some type of protection.<sup>2</sup>

The EPA Ecological Effects Test Guidelines OCSPP 850.1020 recommends for flowthrough studies, “a substrate, such as a bent piece of stainless steel screen, should be placed on the bottom of each test vessel to provide cover for the gammarids.”<sup>1</sup> In addition, the introduction of sectioned PVC pipe substrates to test chambers in a limited number of acute toxicity tests appeared to improve control gammarid survival.

The objective of this trial was to determine the survival of the *G. pseudolimnaeus* during a 96-hour period under static test conditions with the presence of sectioned PVC pipe or bent stainless steel screen substrates.

### EXPERIMENTAL DESIGN

The study was performed based upon procedures in the U.S. EPA Series 850-Ecological Effects Test Guidelines, OCSPP# 850.1020: Gammarid Acute Toxicity Test.<sup>1</sup>

Gammarids were introduced to a negative control (dilution water without a substrate), and test chambers that contain either sectioned PVC pipe substrates (PVC) or bent stainless steel screen substrates (SSS) for 96 hours under static conditions. Eight replicate test chambers were maintained in each group, with five gammarids in each chamber so that a total of 40 gammarids were exposed in each substrate and control group.



Figure 1. Side view, front view, and top-down view of the bent stainless steel screen “tent” (left) and the sectioned PVC pipe substrates (right).



Figure 2. Stainless steel screen and sectioned PVC pipe substrates soaking in dilution water. Substrates soaked for >24 hours prior to use in trial.



Figure 3. One replicate test chamber from the sectioned PVC pipe substrate group (pink), the stainless steel screen substrate group (teal), and the negative control group (gray). All test chambers were indiscriminately placed.

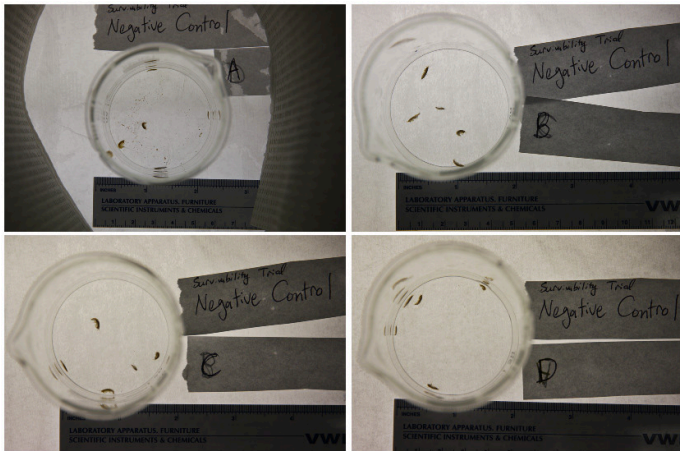
### MATERIALS

#### Test Substrates:

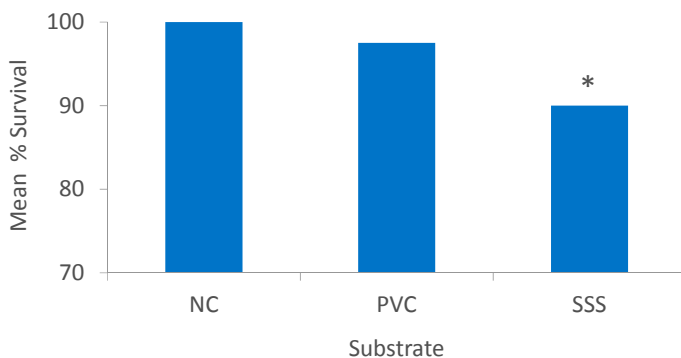
- PVC pipe 1/4” in diameter, cut into ~1 1/4” long segments, and cut in half lengthwise to create tiles. (Figure 1)
- T-316 Stainless Steel 30 x 30 mesh screen, cut into 1 1/4” x 1 3/4” rectangles and bent once down the middle to create a 1 1/4” wide “tent.” (Figure 1)

Substrates were washed, soaked, and rinsed with dilution water prior to test initiation to prevent possible leaching from substrates

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**Figure 4.** Top-down images of transfer containers for replicates A-D of the negative control group. Three images were taken of each transfer container prior to initiation.



**Figure 5.** Mean percent survival of the negative control (NC), sectioned PVC pipe substrate (PVC), and bent stainless steel screen substrate (SSS) groups at test termination. Asterisk denotes significant difference from other groups.

into test chambers (Figure 2).

- **Organism:** Field-collected, mixed-age freshwater amphipod.
- **Dilution Water:** U.V. Sterilized Well Water
- **Test Apparatus:** 2 L glass beakers, filled with ~500 mL of dilution water. Three units of the selected substrate in each chamber. (Figure 3)

### Initiation Procedure

- To achieve uniformity in organism size the gammarids were visually assessed and atypically large and small gammarids were removed from culture containers and not used for

initiation.

- Gammarids were indiscriminately placed into transfer chambers 1-2 at a time until each contained 5 organisms.
- Each transfer chamber was photographed from above (Figure 4) in order to determine initial gammarid size (photographs to be analyzed).
- Gammarids were then introduced using a wide-bore pipette to test chambers already containing substrates as to avoid injuring organisms.

### RESULTS

- The mean percent survival of the gammarids in the SSS Substrate group (90.0%) was significantly less than the mean percent survival of the gammarids in both the PVC Substrate group (97.5%) and NC group (100%) 4,5 (Figure 5.)
- The cumulative percent survival of the PVC Substrate group was not significantly different than that of the NC group 4,5 (Figure 5.)
- All study groups met criteria for an acceptable test.<sup>1</sup>
- Results of this trial suggest that the addition of substrates to test chambers does not improve gammarid survival.
- Results of this trial suggest other factors may attribute to low survival:
  - **Uniformity of organism size (photos to be analyzed)**
  - **Seasonal Effects (time of field collection)**
  - **Unknown age of organisms**

### ACKNOWLEDGMENTS

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### REFERENCES

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