

Abstract

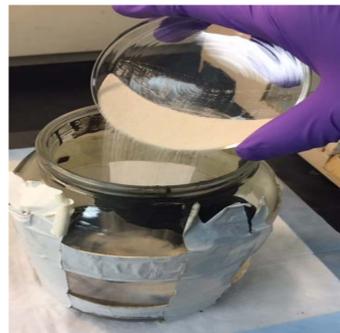
OCSPP guidelines 850.1735 and 850.1740 (spiked whole-sediment 10-day toxicity tests) recommend the use of both a negative control group and a solvent control group when a volatile solvent is used to spike the sediment with the test substance. However, when the solvent is evaporated completely, as recommended in the OCSPP 850.1000 guideline, the Agency will allow the use of only one control (solvent) to be used, if the testing laboratory can demonstrate the functional equivalency of the negative and solvent controls. The EPA suggests using the following four factors when determining equivalence between the negative control and solvent control: (A) Concentration of solvent in the test sediment after evaporation (e.g., analytically determined); (B) Levels of the solvent that are known to affect organism health; (C) The potential for impurities in the solvent and their potential impact on organism health and; (D) Historical organism performance of solvent vs negative controls. Our study will evaluate historical relative performance of the negative control and solvent control, as well as seek to determine the concentrations of the solvent (acetone) that may interfere with the performance of the organisms tested (*C. dilutus*, *H. azteca*, *L. plumulosus*). The study will be conducted as a solvent spiked sediment exposure. The results of this study will establish whether standard testing procedures may result in potential solvent interference, and if not, establish functional equivalency of the two controls, and validate the use of only one control (solvent) during 10-Day sediment testing.

Study Design

- All studies conducted as 10-Day Whole Sediment Exposures following OCSPP guidelines 850.1735 and 850.1740.

Typical Sediment Spiking Technique.

- 10 ml of solvent stock is added to 50g of silica sand and allowed to evaporate for at least 20 minutes. Sand is mixed with a metal spatula during the drying process.
- Once the acetone is evaporated, the test material is left in the sand. The dry sand is then added to the appropriate amount of sediment.



What if the Acetone did not Evaporate?

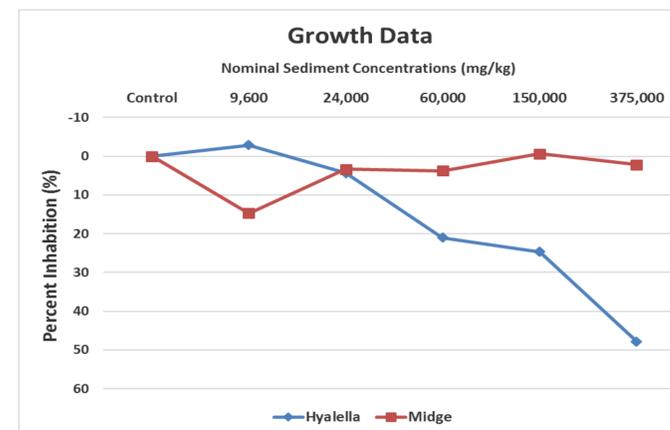
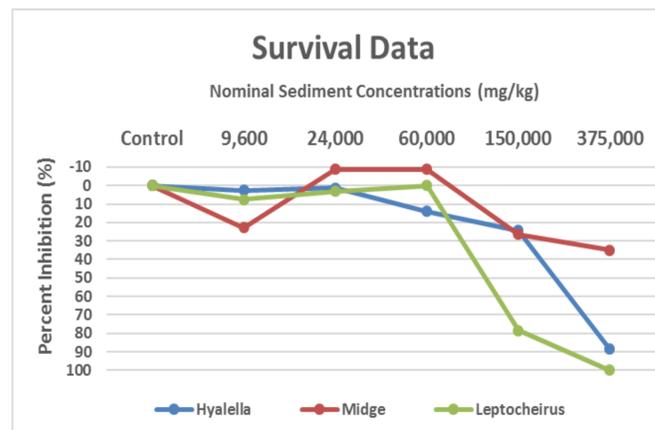
Volume of Acetone Applied (mL)	Density of Acetone (mg/mL)	Wet Weight of Sediment Used (kg)	% Solids of Sediment Used	Sediment Dry Weight (kg)	Approximate Concentration of Acetone in Sediment (mg/kg)	Relevance
10	790	0.75	70%	0.5250	~15,000	Midge/Hyalella Preliminary Exposure
10	790	2.0	70%	1.400	~5,600	Midge/Hyalella Definitive Exposure
10	790	0.75	33%	0.2475	~32,000	Leptocheirus Preliminary Exposure
10	790	2.5	33%	0.8250	~9,600	Leptocheirus Definitive Exposure

Sediment Spiking Technique used for Acetone Exposure

- To ensure the maximum amount of acetone in the exposure system, acetone was mixed directly into the sediment and mixed with a laboratory mixer for 15 minutes.
- The sediment was then allocated to the test vessels immediately, along with the overlying water. The vessels were placed in the exposure system and equilibrated over night prior to study initiation the following day.

Results

Exposure Type	NOEC	LOEC	LC ₅₀ [95% CL]	EC ₅₀
Leptocheirus	60,000	150,000	106,000 [84,000, 216,000]	Not Determined
Hyalella	24,000	60,000	200,000 [174,000, 229,000]	> 375,000
Midge	150,000	375,000	> 375,000	> 375,000

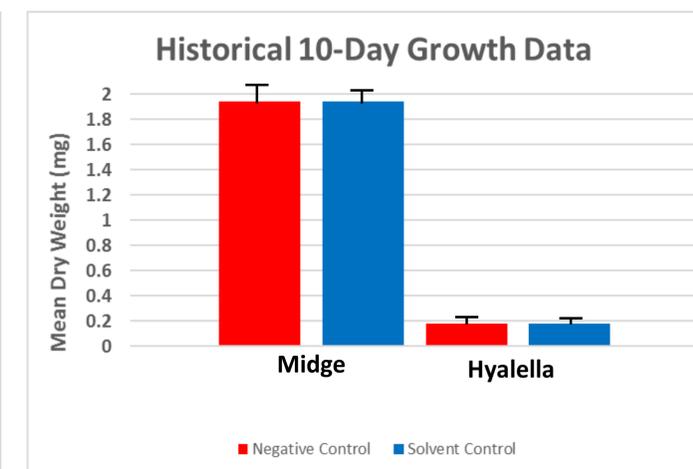
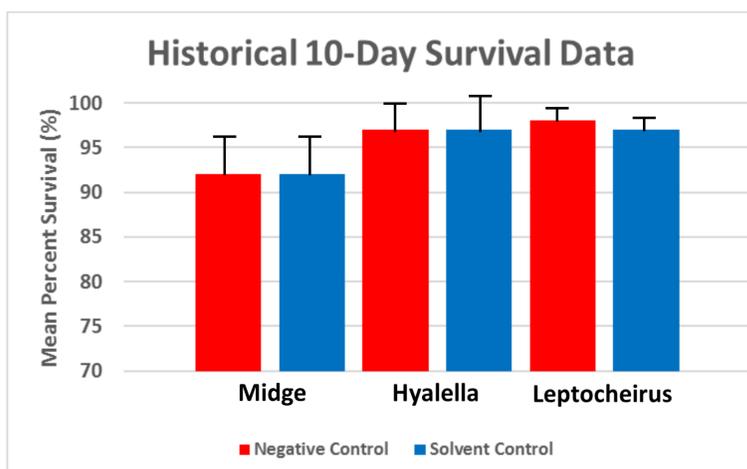


Organism and Solution Observations

- Dissolved Oxygen levels (mg/L) dropped considerably by test day 5 in the treatment levels of the midge and hyalella exposures, and fluctuated above and below acceptability criteria of >2.5mg/L even after the daily volume replacement of the test system was doubled.
- The water quality parameters for the Leptocheirus exposure were normal throughout the duration of the exposure.
- Upon initiation, Hyalella were observed to avoid the sediment surface and remain in the lower third of the water column for the 24,000 and 60,000 mg/kg treatment level, and exhibit complete sediment avoidance and remained high in the water column in the 150,000 and 375,000 mg/kg levels.
- Leptocheirus were observed to burrow in all treatment levels upon initiation except in the 375,000 mg/kg level, where immobility and mortality began within minutes of initiation.
- Observations of the midge behavior appeared to be normal, although the vessels in the 150,000 and 375,000 mg/kg treatment levels were considerably more clear than the other treatment levels and control.

*All results based on nominal sediment concentrations (mg/kg).

Historical Data



Midge Exposures (N=20); Hyalella Exposures (N=21);
Leptocheirus Exposures (N=53)

- There were only two instances where the Negative and Solvent Controls were statistically different.
- Both instances occurred when comparing controls for Midge dry weights.
- In both instances the Control had a higher dry weight than the Solvent Control with the Solvent Control having a 24% and 18% reduction when compared to the Control.

Conclusions and Considerations

- LOEC and LC₅₀ values were both determined to be at least 11 times greater than the hypothetical sediment concentrations used in definitive testing, if the solvent was not evaporated.
- The potential for impurities in the solvent to impact organism performance is negligible as the acetone used in spiking sediments is HR-GC Grade with ≥99.7% assay.
- The historical organism performance (mean survival and mean growth) for all three species (*H. azteca*, *C. dilutus*, *L. plumulosus*) demonstrates the functional equivalency of the negative and solvent controls.
- No lower concentration of acetone was observed to enhance the biological performance, indicating that there should be minimal concern for positive interference.
- As the historical negative control vs. solvent control data demonstrates functional equivalency, and organism sensitivity is greater than 11 times the hypothetical concentration that would be found in the sediment during definitive testing, we recommend the use of only a Solvent Control during 10-Day whole sediment testing.

Acknowledgments

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