TITLE: Assessment Toxicity (28-Day) of Sediments To The Amphipod, *Hyalella azteca* based on Survival and Growth -Project Specific Document. Rev. 6

Approved By:

QA Officer:_____Date:_____

President:_____Date:_____

Revision History:

| Revision Number | Changes | Revised By | Date |
|---|---|---|---|
| 0 | Preparation of SOP | K. A. Simon | 6/00 |
| 1 | Review and update. Correction of light intensity | K. A. Simon | 4/01 |
| 2 | Update, NELAC revisions | K.A. Simon | 07/01 |
| 3 | Clerical Corrections clarifications related to water sources and sieving | K.A. Simon | 09/01 |
| 4 | Review and Update, Addition of NELAC Requirements | S. Dionne | 03/02 |
| 5 | Review and Update | K. A. Simon | 04/04 |
| 6 | Review and Update | K. A. Simon | 07/06 |
| 7 | Review and Update | R. A. McIsaac | 01/09 |
| 7a | Project Specific Document | K. A. Simon | 08/09 |
| 7b | Project Specific Document Rev 1 | K. A. Simon | 09/09 |
| 7c | Project Specific Document Rev 2. Includes modification suggest during 09/15/09 teleconference with Chris Ingersoll, USGS | K. A. Simon | 09/09 |
| 7d | Project Specific Document Rev 3. Includes modification suggest during 09/28/09 teleconference with Chris Ingersoll, USGS | K. A. Simon | 09/29/09 |
| 7e | Addition of pore water ammonia monitoring at the end of the assay. Rev 4 | K. A. Simon | 10/05/09 |
| 7f | Final EPA suggested changes to text. Rev 5 | K. A. Simon | 10/18/09 |
| 7g | Modification of Pore Water Monitoring Parameters in ¶ 5.4.6.3 and 11.21. Rev 6 | K. A. Simon | 10/22/09 |

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## 1.0 Purpose and Applicability

The purpose of this Standard Operating Procedure is to determine the impact, based on survival and growth, of sediments to amphipods exposed under static renewal conditions. The assay involves exposing amphipods to a sediment over a 28 day period. The assay is conducted using guidelines developed by ASTM and is provided in *Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates* (ASTM E1706-05e).

*Hyalella azteca* (Saussure), Amphipoda, have many desirable characteristics of an ideal sediment toxicity testing organism including: relative sensitivity to contaminants associated with sediment, short generation time, contact with sediment, ease of culture in the laboratory, and tolerance to varying physico-chemical characteristics of sediment.

At the end of the 28 day exposure period the amphipods are recovered and counted to establish survival, then dried to establish growth expressed as the average weight/surviving individual and average biomass (total biomass in a replicate divided by the number of organisms in that replicate at the start of the exposure).

This document has been modified to meet project work scope requirements, specified by the U.S. Environmental Protection Agency, for the Lower Passaic River Ecological Risk Assessment. The work is being conducted under contract to Windward Environmental, LLC. Modifications incorporated into the document are related to the salinity of the overlying water used during the assay and culture and acclimation of the test organisms. These modifications are being made to allow the use of a single test species over an extended range of the project where salinity regimes vary beyond the normal range utilized for the species.

## 2.0 Definitions

Overlying Water: The water placed over sediment in a test chamber during a test.

Reference Sediment: A whole sediment near an area of concern used to assess sediment conditions exclusive of material(s) of interest. The reference sediment may be used as an indicator of localized sediment conditions exclusive of the specific pollutant input of concern. Such sediment would be collected near the site of concern and would represent the background conditions resulting from any localized pollutant inputs as well as global pollutant input. This is the manner in which reference sediment is used in dredge material evaluations.

Reference-Toxicity Test: A test conducted with reagent-grade reference chemical to assess the sensitivity of the test organisms. Deviations outside an established normal range may indicate a change in the sensitivity of the test organism population. Reference-toxicity tests are most often performed in the absence of sediment.

Pore water: Water located in spaces between grains of sediment.

Sediment: Particulate material that usually lies below water. Formulated particulate material that is intended to lie below water in a test.

Whole Sediment: Sediment and associated pore water which have had minimal manipulation. The term bulk sediment has been used synonymously with whole sediment.

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## 3.0 Applicable Documents/References

ASTM. 2009. *Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates.* E 1706-05e, West Conshohocken, PA.

U.S. EPA. 2000. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*. Second Edition. EPA/R-99/064. March 2000.

ESI SOP# QA-1203-R5-2003: Preparation of Daphnia Food

ESI SOP#QA-1339-R4-2008: Collection of Sediment Pore Water Samples

ESI SOP#QA-1219-R0-2009: Use and Operation of the YSI Model 556 Multi Probe System meter

ESI SOP#QA-1114-R3-2003: Conduct of Reference Toxicant Assays

ESI SOP#QA-1341-R1-2009: Sulfide Analysis by Titration

ESI SOP#QA-1320-R6-2009: Statistical Analysis of Acute and Chronic Exposure Bioassay Data

ESI SOP#QA-1309-R4-2009: Computation of Hardness by Calculation Method

ESI SOP#QA-1326-R6-2009: Alkalinity by Lachat using the Automated Phenate Method

ESI SOP#QA-1325-R8-2009: Ammonia by Lachat

ESI SOP#QA-1336-R4-2007: Measurement of Total Organic Carbon using the Phoenix 8000 Analyzer

## 4.0 Materials and Apparatus

Test animals
Beakers, 400 mL, drilled and screened for flow through
Incubator/water bath capable of maintaining a temperature of 23 ±1°C
Dissolved oxygen meter, pH meter, conductivity meter, temperature logger
Light Meter
Balance, capable of reading 0.01 mg
Drying Oven, 60°C
Components for artificial sediment - fine sand, organic material
Sieves
YCT Food (See SOP #1203)
Refractometer

## 5.0 Methods/Procedures

### 5.1 Test Material

5.1.1 Test substances will be clearly identified as to source, collection date, period of collection and description of collection methods. The laboratory will identify sample storage procedures and maintain receipt, storage, usage and disposition records.

5.1.2 Project site sediments will be stored at 2-4°C in the original shipping container.

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Maximum holding time is 8 weeks. Once open, a sample container with remaining sample will be purged using an inert gas prior to storage. Inert gas will be either nitrogen or argon.

5.1.3 Prior to test preparation, the sediment is warmed to 23°C.

5.1.4 Prior to use, sediment total organic content (loss on ignition) is measured for each sediment sample.

5.1.5 Prior to use in the assay, pore water salinity will be measured using a refractometer or salinometer, as appropriate. Samples having a pore water salinity of ≤5‰ will be identified as being tested using freshwater as the overlying water while all samples having a pore water salinity of >5‰ will be identified as being tested using a 10‰ salinity overlying water.

5.2 Test Organisms

5.2.1 Healthy amphipods from the same source and age are used in the test. Amphipods will be between 7 and 8 days old.

5.2.2 Confirmation of species is provided by the supplier. If not provided, ESI will use the services of either the zoology department of the University of New Hampshire or the taxonomy group of Normandeau Associates, Bedford, NH to confirm the species. Organisms maintained by ESI will be from cultures of the confirmed species.

5.2.3 Pretest observation data concerning the source, handling procedures, disease treatment (if any), health, feeding, mortality and mean dry weight of test animals will be recorded and reported. Mean dry weight of organisms at the start of the assay are based on that of 40 organisms measured in groups of 10. Growth, dry weight, of similar aged adult amphipods will be monitored in both freshwater and saltwater. Acclimated amphipod cultures will be monitored prior to use in the assays to provide a qualitative measurement of growth in the two culture systems. Young production in all cultures will be qualitatively compared to determine if there are substantial differences in reproduction between freshwater cultures and saltwater-acclimated cultures.

5.2.4 Organisms used in the assays will be selected from cultures of appropriate salinity. Salinity levels utilized will be either freshwater, <0.5‰, or 10‰, depending on the pore water salinity of an individual sample.

5.3 Exposure Conditions

Exposure conditions and monitoring requirements detailed in the following paragraphs are the minimum requirements specified by ESI for this assay.

5.3.1 Sediments are placed in vessels, overlying water is added and the samples are allowed to settle overnight before organisms are added to the test.

5.3.2 A laboratory control will be established for both fresh and salt water sample sets.

5.3.3 Assays are conducted in a static renewal mode. Overlying water is renewed on a

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daily basis, at the rate of two (2) volume additions per day.

5.3.4 Mean water temperature is 23 ±1°C. Maximum temperature deviations should not exceed ±3°C of the specified value at any time.

5.3.5 The photoperiod is set to 16 hours light : 8 hours dark. Light intensity is 100 to 1000 lux from wide spectrum fluorescent fixtures.

5.3.6 The test vessels are 400 mL beakers containing 100 mL of sediment and 225 mL of water.

5.3.7 Sediment for the laboratory control treatment will be a formulated sediment. Formulated sediments will be prepared, by weight, as follows:

| | | |
|---|---|---|
| Fine grained sand | - | 95% |
| Organic matter | - | 5% (approximate) |

Sand used will be an equal mix of F-75 and F-65 unground quartz (silica) sand provided by New England Silica

The preferred source for organic matter in the artificial sediment is the organic matter recovered from either midge larvae or amphipod cultures. The material is screened to remove large matter and then autoclaved for a half hour to insure sterility. Organic content of the control sediment will be adjusted prior to the start of the assay to be representative of that of the project site sediments.

5.3.8 The type of the overlying water will be determined based on individual sample pore salinity. Samples with a pore water salinity of ≤5‰ will use freshwater as the overlying water while all samples having a pore water salinity of >5‰ will use an overlying water of 10‰.

5.4 Study Conduct

5.4.1 The animals are exposed for 28 days to the test sediment and to the untreated control sediment, under static renewal conditions. Overlying water renewal is equal to two (2) volume additions per day.

5.4.2 A representative sample is obtained from sediment provided. Test sediments are homogenized and 100 mL placed in each test chamber. Sediments may be dry-sieved through a ≥3 mm screen to remove debris. 225 mL of water is added to the test chamber and allowed to settle overnight. Aeration is provided to each test chamber if the dissolved oxygen levels are <2.5 mg/L. Aeration is designed to provide approximately 1 bubble/second and set so as to not disturb the sediment surface. Prior to the addition of the test organisms, any floating detritus is removed from the surface of the water.

5.4.3 Overlying water.

5.4.3.1 Overlying water for samples having a pore water salinity of ≤5‰ will be moderately hard reconstituted laboratory water prepared as specified in Table 1 (page 26) of EPA-600/4-91/002 mixed with a natural surface water

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on a 50/50 basis. Surface water will be filtered, 100µm, prior to addition to the reconstituted water. Conductivity of the overlying water will be documented and reported on a daily water quality sheet prior to use in the assay.

5.4.3.2 Overlying water for samples having a pore water salinity of >5‰ with be saltwater having a salinity of 10‰. The 10‰ saltwater will be prepared by diluting natural saltwater with deionized water. Natural saltwater will be filtered, 100 µm, prior to dilution. Salinity and conductivity of the overlying water will be documented and reported on a daily water quality sheet prior to use in the assay.

5.4.3.3 Daily overlying water renewals will be made using a systems developed by ESI. The system provides equal flow to a specified number of test chambers, variable based on test chamber size. Flow to each chamber is controlled by fixed diameter orifice. The total amount of overlying water to be exchanged is computed and added to the unit. Flow starts when water is added to the system on Day -1. Water is added to the system so that all water required for the renewal is added within 15 seconds. If the system can not hold sufficient water for the 2 volume additions then a second addition to each chamber will be made. Flow rates will be set prior to the start of an assay and maintained constant throughout the assay. Flow rates will be set to minimize disturbance of and re-suspension of the sediment surface and so as not to cause water levels to exceed that of the overflow in the test chamber.

5.4.4 Test organisms will be 7 - 8 days old at the start of the assay.

5.4.5 Each treatment group will consist of 80 animals, 10 animals per test vessel. The animals are randomly assigned to the test vessels on day 0.

5.4.6 Measurement of Water Characteristics

5.4.6.1 Prior to renewal, temperature, dissolved oxygen, specific conductance, salinity and pH are measured daily during the test, in a surrogate test chamber for each treatment. Surrogate chambers will be treated in the same manner as all other replicates and will receive test organisms and will be fed on the same schedule.

5.4.6.2 Temperature is recorded hourly using a data-logger placed in a separate sealed test chamber containing dry control sediment.

5.4.6.3 Alkalinity, hardness and ammonia of the overlying water are measured in the overlying water of a surrogate test chamber for each treatment at the start of the test, and weekly thereafter. TOC of the overlying water is measured in a surrogate at the start and end of the assay. Pore water salinity, pH, ammonia, hardness and alkalinity will be measured in a subsample of each sediment sample prior to the start of the assay.

5.4.6.4 At the end of the assay, pore water ammonia levels will be determined for each sample treatment. Pore water for this analysis will be collected from the surrogate test chamber.

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5.4.7 Amphipods are fed 1.0 mL of YCT food per vessel, solids content 1800 mg/L, daily. Feeding will be suspended if fungus is noted forming on more than approximately 25% of the sediment surface.

5.4.8 The assay is terminated on day 28. Sediments are placed on an appropriately sized screen and gently washed with fresh water to remove sediment. Material remaining on the screen is examined to recover remaining amphipods. Any amphipod that shows signs of movement is considered alive, and counted.

5.4.9 All surviving amphipods from an individual replicate are rinsed with lab water, to remove any detritus, placed on a tared pan and dried at 60°C for 24 hours. Pans are allowed to cool to room temperature in a desiccator and weighed to the nearest 0.01 mg.

## 6.0 Quality Control Requirements

6.1 Reference Toxicant Evaluation

A reference toxicant evaluation should be conducted with each series of assays, ESI SOP#1114-R3-2003. The reference toxicant shall be a 96-hour 'water only' test conducted with cadmium chloride. A separate reference toxicant will be conducted for organisms maintained in each water type, fresh and salt.

6.2 Interferences

Living organisms present in the sample may compete with the amphipods or may be predators, reducing overall survival. This impact may be mitigated by sieving samples prior to testing.

## 7.0 Calculations/Reporting

7.1 Data Analysis

Survival and growth data from each treatment will be subjected to analysis of variance (ANOVA) to determine if significant differences exist between treatments and the control. Statistical evaluations will be made with CETIS® software using appropriate standard statistical models.

7.1.1 Prior to statistical analysis, the survival and growth data will be reviewed to determine the presence of outliers. If outliers are found, an explanation must be sought. If a reasonable source for the deviation is found, the value may be excluded from further analysis. If no explanation is found, the analysis should be performed both with and without the questionable data point and both sets of results reported. All data sets are evaluated to determine sample variance homogeneity and normality. Those data sets meeting the criteria for normality and homogeneity are evaluated with parametric statistical models, while those that do not meet both criteria are evaluated using non-parametric models.

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7.1.2 Endpoints evaluated are: survival and growth (dry weight) at day 28.Growth endpoints evaluated include both dry weight and dry biomass. Dry weight is determined as the total dry weight divided by the number of surviving/recovered organisms. Dry biomass is determined as total dry weight divided by the number of organisms used per replicate at the start of the assay.

7.1.3 Statistical comparisons for each sample site will be made against the laboratory control treatment plus each project reference site or as otherwise specified. Project reference sites may also be compared to one another.

7.2 Reporting

Reports generated from this study will include: summarization of collection and transportation information (as provided), methods and materials, test organism history, test conditions, documentation of variations from the proposed work scope, and results and data analysis. Copies of all statistical printouts and raw data are attached as an appendix to the report.

## 8.0 Corrective Actions

8.1 Acceptability Criteria

8.1.1 The amphipod assay is considered acceptable if environmental parameters (temperature, dissolved oxygen, salinity, pH, alkalinity and hardness) fall within the ranges specified. Survival in the control sediments after 28 days will be $\geq$80%.

8.1.2 Criteria specified in Section 11 will be met.

8.2 If survival fails to meet the minimum value specified by the protocol, the client will be notified and the test restarted.

8.3 If water quality values fall outside study limits the Laboratory Manager, using sound scientific practice, will determine if the study requires repeating or the data is allowed to be accepted. The client will be notified, the results reviewed and a final determination made as to the acceptability of the data.

8.4 In the event that an element of the assay falls outside acceptable limits, or there is a change in the protocol, a Corrective Action Report must be initiated and completed.

## 9.0 Health and Safety

9.1 As with all samples, gloves and safety glasses should be worn when handling sediment samples and chemicals. It is advisable to wear a lab coat to protect clothing.

9.2 At the end of an assay excess sample material and material used in the assay will be disposed of appropriately. Material may be returned to the client, or air dried and placed in an appropriate container for disposal at an approved disposal facility. If the material is classified as non-hazardous, the material may be disposed in an appropriate waste container.

9.3 Assays and sample disposal will be conducted using procedures to minimize potential pollution of surface and ground waters, soils and sediments.

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**10.0 Responsibilities**

10.1 It is the Lab Manager's responsibility to ensure analysts performing this procedure are properly trained and the training is documented in their training file. The analyst is responsible for following the procedures outlined in this SOP.

10.2 Prior to any staff member working unsupervised on a testing procedure, they must be certified by the Laboratory Manager. Certification will include reading this and associated SOPs, review of the primary literature and participation in similar procedures under the direct supervision of a trained staff member. Certification will be based upon a review of the persons' demonstrated abilities.

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## 11.0 Summary of Test Conditions

| | | |
|---|---|---|
| 1. | Test Mode: | Static renewal |
| 2. | Overlying Water Renewal: | 2 Volume additions per day |
| 3. | Temperature: | Mean 23 ±1°C with no values exceeding limits of ±3°C of the mean |
| 4. | Photoperiod: | 16 hr light:8 hr dark |
| 5. | Light Source: | Wide-spectrum fluorescent |
| 6. | Light Intensity: | Approximately 400 to 600 Lux |
| 7. | Test Chamber: | 400 mL beakers |
| 8. | Test Solution Volume: | 100 mL sediment; 225 mL overlying water |
| 9. | Organisms/Chamber: | 10 |
| 10. | Replicates/Treatment: | 8 |
| 11. | Organisms per Treatment: | 80 |
| 12. | Age of Organisms: | 7 - 8 days |
| 13. | Food Source: | YCT mix; 1800 mg/L solids |
| 14. | Feeding Regime: | 1.0 mL YCT daily |
| 15. | Overlying Water: | For samples having a pore water salinity of ≤5‰ a mix of moderately hard synthetic water (hardness of 40 - 100 mg/L, alkalinity of 20 - 70 mg/L) and natural surface water. Hardness and alkalinity of overlying water shall not vary by 50% during the assay. For samples having a pore water of >5‰ the overlying water will be 10‰ salt water |
| 16. | Aeration: | 1 bubble/second, if dissolved oxygen falls below 2.5 mg/L |
| 17. | Test Duration: | 28 Days |
| 18. | Endpoint: | Mortality and growth (dry weight to 0.01 mg and/or length to 0.01 mm) |
| 19. | Sample Holding Time: | 8 weeks at 2-4°C after collection from the field |
| 20. | Acceptability: | Control survival equal to or exceeding 80% and measurable growth in control treatment. Hardness, and alkalinity of overlying water not to vary by 50% during assay |
| 21. | Analytical Support: | Daily measurement of temperature, dissolved oxygen, specific conductance, salinity and pH in overlying water of one replicate of each treatment. Measurement of alkalinity, hardness and ammonia in overlying water of one replicate of each treatment at the start and end of the assay plus weekly during the assay. Measurement of salinity, pH, ammonia, hardness and alkalinity in water isolated from a sub-sample of whole sediment prior to the start of the assay. Measurement of ammonia in pore water isolated from sediment in the surrogate test chamber at the end of the assay. Hourly temperature measurement in a separate test vessel |

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**12.0 Organism Culture - 10‰ Salinity**

*Hyalella azteca* will be acclimated to 10‰ salinity prior to use in assays requiring elevated overlying water salinity. Amphipod cultures will be maintained at the 10‰ salinity for a minimum of 1 month prior to use in the assays. Guidance for salinity adjustment and salinity tolerance was obtained, in part, from Chris Ingersoll. Dr. Ingersoll is the Branch Chief, Toxicology, at the U.S. Geological Survey Columbia Environmental Research Center. Based on communications with Dr. Ingersoll, the following acclimation and culture protocol will be responsive to the requirements of this program. Historical data from work conducted by the Columbia Environmental Research Center staff indicate that *H. azteca* cultured at 10‰ exhibited no negative responses when used in short-term exposure assays.

12.1 Acclimation

12.1.1 Acclimation from freshwater, culture water of<0.5‰ salinity, to 10‰ should be at a rate of salinity change of no more that 2‰ per day.

12.1.2 Salinity shall be adjusted by the addition of natural seawater.

12.1.3. Salinity will be measured using a refractometer or salinometer.

12.1.4 Daily acclimation will be maintained.

12.2 Culture

12.2.1 Once at the appropriate salinity, 10‰, organism culture techniques will follow standard protocol with respect to feeding, culture renewal and general maintenance.

12.2.2 Salinity will be measured on a regular basis as part of routine culture maintenance.

12.3 Additional Support

12.3.1 Once the cultures have become acclimated to the target salinity a series of reference toxicant assays will be conducted to establish a database for the acclimated organisms.

12.3.2 Once the cultures are of sufficient age, they will be examined weekly to establish that reproduction is occurring.

12.3.3 When a culture is started, a representative group of amphipods will be randomly selected and their dry weight determined. After 28-days, a group of adults will be removed from the culture for the purpose of obtaining dry weights. Data will be evaluated to determine that growth has occurred and compared to historical growth data associated with routine cultures.

Reference

C. Ingersoll. 2009. Personal communication. Branch Chief, Toxicology, U.S. Geological Survey Columbia Environmental Research Center.

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