TITLE: Acute Toxicity of Sediments To Midge Larvae, *Chironomus dilutus* - Project Specific Document

Approved By:

QA Officer:_____Date:_____

President:_____Date:_____

Revision History:

| Revision Number | Changes | Revised By | Date |
|---|---|---|---|
| 1 | Review and Update | K. A. Simon | 01/93 |
| 2 | Review and Update | K. A. Simon | 04/95 |
| 3 | Review and Update | K. A. Simon | 02/97 |
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| 6 | Update based on new ASTM protocol | K. A. Simon | 04/00 |
| 7 | Update to include NELAPC material and correct light intensity | K. A. Simon | 05/01 |
| 8 | Review and Update, Addition of NELAC Requirements | S. Dionne | 03/02 |
| 9 | Update and Review | K. A. Simon | 04/04 |
| 10 | Update and Review | K. A. Simon | 07/06 |
| 11 | Update and Review | K. A. Simon | 03/08 |
| 12 | Update | R.A. McIsaac | 01/09 |
| 12a | Project Specific Document with modifications based on comments by Dr. Chris Ingersoll, USGS, to the 28-day *Hyalella azteca* SOP that generally apply to sediment bioassay testing. | N. Roka | 10/14/09 |
| 12b | Editorial corrections | R. A. McIsaac | 10/15/09 |
| 12c | Revised based on comments from USEPA and CPG (Cooperating Parties Group) for the Lower Passaic River Restoration Project | K. Simon | 11/18/09 |

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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 midge larvae exposed under static renewal conditions. The exposure period for this assay is 10 days. The assay is completed using guidelines developed by ASTM and U.S. Environmental Protection Agency (USEPA) and is provided in *Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates* (ASTM E 1706-05e) and *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*. Second Edition (USEPA).

At the end of the 10-day exposure period the midge larvae are recovered, enumerated and dried to establish survival and growth. Growth is 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 for the Lower Passaic River Ecological Risk Assessment. The work is being conducted under contract to Windward Environmental, LLC.

## 2.0 Definitions

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

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.

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.

Pore Water: Water located in the spaces between grains of 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 of appropriate age
Beakers, 400 mL, drilled and screened
Incubator/ waterbath, capable of maintaining a temperature of 23 ±˚C
Dissolved oxygen meter, pH meter, conductivity meter, temperature logger
Light meter
Balance capable of reading 0.01 mg
Drying Oven, 60 ±5˚C
Muffle furnace, 550˚C
Tetramin® Fish Food
Components for artificial sediment - fine sand and organic material
Sieves
Refractometer
Inert gas - Nitrogen or Argon for sample storage

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## 5.0 Methods/Procedures

5.1 Test Material

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

5.1.2 Project site sediments are stored at 2-4˚C in the original shipping container. Maximum holding time is 8 weeks. Once open, headspace within a sample container with remaining sample is purged using an inert gas prior to storage. Inert gas can 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 in the assay, 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 is measured using a refractometer or salinometer, as appropriate.

5.1.6 Prior to use in the assay, pore water ammonia, pH, hardness and alkalinity is measured in a sub-sample of each sediment sample.

5.2 Test Organisms

5.2.1 Healthy larvae from the same source and age, second to third instar, with at least 50% of the organisms having achieved third instar stage, are used in the tests. Midge larvae are considered to be at the third instar stage between 8 and 10 days after the egg masses hatch.

5.2.2 Confirmation of species is provided by the supplier. If not provided, ESI will use the services of a qualified taxonomist to confirm the species (e.g., the zoology department of the University of New Hampshire, the taxonomy group of Normandeau Associates, Bedford, NH). 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 and mortality of test animals are recorded and reported.

5.2.4 Initial weight is obtained on each batch of organisms tested by randomly selecting a minimum of 20 organisms from the pool to be used in the assay. The organisms are rinsed, placed on tared weigh pans and dried at 60˚C for 24 hours. The dried organisms are then cooled in a dessicator and weighed to the

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nearest 0.01 mg to obtain mean initial dry weight.

5.3 Exposure Conditions

Exposure conditions and monitoring requirements detailed in the following paragraphs are the minimum requirements specified by ESI for this assay. Notify the project manager prior modifying exposure conditions or monitoring requirements.

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 Assays are conducted in a static renewal mode. Overlying water is renewed at the rate of two (2) volume additions per day.

5.3.3 Mean water temperature is maintained at 23 ±1˚C. The daily mean temperature must be within ±1˚C of the specified temperature and maximum temperature deviations should not exceed ±3˚C of the specified value at any time.

5.3.4 The photoperiod is set for 16 hours light : 8 hours dark. Light intensity is 400 - 1000 lux from wide spectrum fluorescent fixtures.

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

5.3.6 Sediment for the laboratory control treatment is a formulated sediment. Formulated sediments are prepared, by weight, as follows:

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

Sand used consists of 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 ensure sterility. Organic content of the control sediment will contain approximately 5% organic matter to be similar in organic content to project site sediments based on historical data for surface sediments collected from the study area between 2005 and 2008 (information provided by the CPG).

5.3.7 Overlying water is natural surface water collected by ESI from a location with a documented history of acceptable use in culturing and conducting tests with *C. dilutus*. The natural surface water is filtered using a 100µm screen prior to use. Basic properties of the overlying water, such as alkalinity, hardness, ammonia and specific conductance should not vary by more than 50% between the start and end of the assay.

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5.4 Study Conduct

5.4.1 Test organisms are exposed to the test sediment(s) and untreated control sediment for 10 days, under static renewal conditions.

5.4.2 A representative sample will be obtained from the homogenized test sediment and 100 mL of sediment is placed in the test chamber. Then 225 ml of overlying water is added to the test chambers and allowed to settle overnight. 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 is natural surface water (filtered using a 100µm screen). Conductivity of the overlying water is documented and reported on a daily water quality sheet prior to use in the assay.

5.4.3.2 Daily overlying water renewals are conducted using a system 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 cannot 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 and re-suspension of the sediment surface and to prevent water levels from overflowing the test chambers.

5.4.4 Test organisms are second to third instar, with 50% of the organisms having reached the third instar stage, at the start of the assay. Prior to use, test organisms should be held for a minimum of two hours under similar conditions to those used in the assay.

5.4.5 Each treatment group will consist of 80 animals with 10 animals placed randomly in each test vessel.

5.4.6 Measurement of Water Characteristics

5.4.6.1 Prior to renewal, dissolved oxygen, pH, conductivity and temperature are measured daily during the test in one surrogate test chamber for each treatment. Surrogate chambers are treated in the same manner as all other replicates including the addition of test organisms and feeding schedule.

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5.4.6.2 Temperature in the water baths used to maintain constant temperature in the test chambers is measured on an hourly basis using a data-logger placed in a test chamber containing dry sediment. The chamber is weighted to keep it from floating in the water bath.

5.4.6.3 Alkalinity, hardness and ammonia are measured in the overlying water in a surrogate test chamber for each treatment at the start and end of the assay.

5.4.6.4 At the end of the assay, pore water ammonia and pH levels are determined for each sample treatment. Pore water for these measurements are collected from a surrogate test chamber.

5.4.7 Midge larvae are fed 1.5 mL of 6.0 g/L Tetramin® flake fish food suspension daily. Feeding is suspended if fungus is noted forming on more than approximately 25% of the sediment surface.

5.4.8 The assay is terminated on Day 10 at the conclusion of the full 10-day exposure period. Test sediment is removed from the test vessel, placed on an appropriately sized screen and washed with water. Midge larvae are rinsed, collected, counted, and set aside for determination of survival and growth (ash free dry weight).

5.4.9 Surviving larvae from each individual replicate are rinsed again with lab water, to remove any detritus, then placed on a tared pan and dried at 60˚C for 24 hours. Pans are cooled to room temperature in a desiccator and weighed to the nearest 0.01 mg. (This is recorded as dry weight.) Pans are then placed in a muffle furnace and organisms are ashed for two hours at 550˚C. Pans are cooled to room temperature in a desiccator and weighed to the nearest 0.01 mg. (This is recorded as ash weight.) The tissue mass of the larvae is determined as the difference between the weight of the dried larvae plus pan and the weight of the ashed larvae plus pan.

## 6.0 Quality Control Requirements

6.1 Acceptance Criteria

6.1.1 Mean control survival ≥70%, mean ash free dry weight ≥0.48 mg per individual. Hardness, alkalinity and ammonia levels of overlying water stock not to vary by 50% during assay

6.1.2 All criteria from Section 11: Summary of Test Conditions, must be met.

6.2 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

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conducted with cadmium chloride.

6.3 Interferences

Living organisms present in the sample may compete with the midge larvae or may be predators, reducing overall survival.

## 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 are 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. See SOP QA-1320 - "Statistical Analysis of Acute and Chronic Exposure Bioassay Data"

7.1.2 Endpoints to be evaluated include; survival and growth, measured as ash free dry weight and ash free dry biomass, after10 days of exposure.

7.1.3 Statistical comparisons for each sample site will be made against the laboratory control treatment.

7.2 Reporting

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

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## 8.0 Corrective Actions

8.1 Acceptability Criteria. The midge assay is considered acceptable if:

8.1.1 Environmental parameters (temperature, dissolved oxygen, conductivity, pH, alkalinity and hardness) fall within the ranges specified. (i.e., hardness and alkalinity of overlying water stock does not vary by 50% during the assay).

8.1.2 Mean control survival is $\geq$70%

8.1.3 Mean ash free dry weight is $\geq$0.48 mg per individual in the controls.

8.1.4 Criteria specified in Section 11 are met.

8.2 If survival fails to meet the minimum value specified by the protocol the client will be notified and the test restarted. Water quality data are reviewed when collected and any necessary steps are taken to ensure that values approaching, or outside study limits, are corrected. In such cases the Project Manager will be notified.

8.3 If water quality values fall outside study limits the Project Manager, using sound scientific practice, will determine if the study requires repeating or the data are 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 the process, excess sample material and material used in the collection process 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 Sample disposal will be conducted using procedures to minimize potential pollution of surface and ground waters, soils and sediments.

## 10.0 Responsibilities

10.1 It is the Project Manager's responsibility to ensure analysts performing this procedure are

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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 Project Manager. Certification will include reading this and associated SOP's, 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

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| 1. | Test Mode: | Static renewal |
| 2. | Water Renewal: | 2 volume additions per day |
| 3. | Temperature: | Mean 23 ±1˚C , 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 -1000 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: | Second to third instar with ≥50% of the midge larvae at third instar |
| 13. | Food Source: | 6.0 g/L Tetramin® flake fish food suspension |
| 14. | Feeding Regime: | 1.5 mL of flake fish food suspension per day |
| 15. | Dilution Water: | 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 on a 50/50 basis. |
| 16. | Aeration: | If dissolved oxygen falls below 2.5 mg/L, then all vessels are aerated at 1 bubble/second delivered 2 cm above the sediment interface. |
| 17. | Test Duration: | 10 days |
| 18. | Endpoint: | Mortality and growth, as ash free dry weight and ash free dry biomass (to 0.01 mg) |
| 19. | Sample Holding Time: | 8 weeks at 2-4˚C after collection from the field. Displace headspace with inert gas after opening sample container. |
| 20. | Acceptability: | Control survival equal to or exceeding 70%; mean ash free dry weight of at least 0.48 mg per individual; hardness, alkalinity and ammonia of overlying water not to vary by more than 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 in one replicate of each treatment at the start and at the end of the assay. Measurement of salinity, ammonia, pH, hardness and alkalinity in pore water isolated from a sub-sample of whole sediment from each treatment at the start of the assay. Measurement of ammonia and pH in pore water isolated from sediment in the surrogate test chamber for each treatment at the end of the assay. Hourly temperature measurement in a separate test vessel. |

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