



PORTLAND HARBOR RI/FS
**ROUND 2A QUALITY ASSURANCE
PROJECT PLAN ADDENDUM**

DRAFT

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This document is currently under review by US EPA and its federal, state, and tribal partners, and is subject to change in whole or part.

Prepared for:
The Lower Willamette Group

Prepared by:
Striplin Environmental Associates, Inc.

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LIST OF ACRONYMS

ACG	Analytical Concentration Goals
AG	Amber Glass
ARI	Analytical Resources, Inc.
ASTM	American Society for Testing and Materials
CLP	Contract Laboratory Program
CRITFC	Columbia River Inter-Tribal Fish Commission
CVAA	Cold Vapor Atomic Absorption
DQO	Data Quality Objective
EPA	Environmental Protection Agency
EQulS	Environmental Quality Information Systems
FSP	Field Sampling Plan
HDPE	High Density Polyethylene
ICP	Inductively Coupled Plasma
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LCS	Laboratory Control Sample
LVI	Large Volume Injector
MRL	Method Reporting Limits
NAS	Northwestern Aquatic Sciences
PAHs	Polycyclic Aromatic Hydrocarbons
PARCC	Precision, Accuracy, Representativeness, Completeness, and Comparability
PCBs	Polychlorinated Biphenyls
QA	Quality Assurance
QC	Quality Control
QAPP	Quality Assurance Project Plan
RI/FS	Remedial Investigation/Feasibility Study
RSD	Relative Standard Deviation
SEA	Striplin Environmental Associates
SVOC	Semivolatile Organic Compounds

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1.0 QAPP ADDENDUM

This quality assurance project plan (QAPP) addendum is intended to be used in conjunction with the EPA-approved *Portland Harbor Round 1 Quality Assurance Project Plan, Final Report, November 22, 2002* (SEA 2002) which was developed for the Portland Harbor Remedial Investigation/Feasibility Study (RI/FS). All elements of the EPA-approved, project-specific QAPP are applicable to Round 2A and subsequent phases of analyses. This QAPP addendum was prepared to specifically address the chemical analysis of water samples and biological testing of sediment samples that will be conducted in Round 2A, as well as any subsequent phases of the project.

2.0 CHEMICAL ANALYSES

2.1 SAMPLE CONTAINERS, HOLDING TIMES, AND PRESERVATION

Detailed procedures for collection and handling of water samples are specified and documented in the FSP. Table 1 summarizes sample containers, sample holding times, preservation requirements, and the sample size required for laboratory analysis.

2.2 ANALYTICAL METHODS

The analytical methods, laboratory method reporting limits (MRLs), and analytical concentration goals for water samples are summarized in Table 2. The laboratory MRLs were developed to correspond with end uses of the data. Actual MRLs reported by a laboratory may differ from the goals, depending on the sample matrix. The analytical methods for water samples, listed in Table 3, are consistent with the methods for sediment and tissue from the Round 1 EPA-approved QAPP (SEA 2002). Any modifications to the methods referenced in Table 3 that are not included in the laboratory SOPs (included in the EPA-approved Round 1 QAPP) will be included in the laboratory case narrative for the associated data package and discussed in the data validation report. QA/QC sample control limits are provided in Table 4.

As required in the EPA-approved QAPP (SEA 2002) for Round 1, the laboratory deliverables will be consistent with the requirements of Contract Laboratory Program data packages, and electronic data will be provided by the chemistry laboratories in the format specified for import into the EQuIS data management system.

2.2.1 Metals

Metals analyses will be conducted by inductively coupled plasma atomic emission spectrometry (ICP-AES) (EPA Method 6010B) (EPA 1998), inductively coupled plasma mass spectrometry (ICP-MS) (EPA Method 6020), and cold vapor atomic absorption (CVAA) (EPA Method 7040A), as specified in the methods referenced in Table 3. EPA Method 6020 will be modified by concentrating the extract by a factor of two to achieve the MRLs for cadmium and lead.

2.2.2 Semivolatile Organic Compounds

The analysis for semivolatile organic compounds (SVOCs) will be conducted according to EPA Method 8270C. The SVOC compound list for the project is included on Table 2. To meet the project data quality objectives, the laboratory will employ the following procedures:

- A large volume injector (LVI) will be used for this analysis to allow the laboratory to achieve the MRLs using a 1-liter sample for extraction
- Tentatively identified compounds will be reported.

2.2.3 Chlorinated Pesticides and PCB Aroclors®

Chlorinated pesticides and PCB-Aroclors® will be analyzed according to EPA Methods 8081A and 8082. Sample extracts will be subjected to cleanup techniques listed in the referenced methods, as necessary, to achieve the project MRLs. For chlorinated pesticide analysis, ARI will follow the Manchester extraction procedure, which requires 3 liters of sample for extraction and reduction of the final extract volume to achieve the project-specific MRLs. Alternatively, a LVI may be used by Analytical Resources, Inc. (ARI) for chlorinated pesticide and/or PCB Aroclors® analysis to achieve the MRLs using a 1-liter sample for extraction. If the chlorinated pesticide analysis is conducted using the Manchester procedure, three liters of water will be collected for chlorinated pesticide analysis. If the LVI is not used for PCB Aroclor® analysis, 3 liters of water will also be collected for PCB Aroclor® analysis.

2.2.4 Herbicides

Herbicides will be analyzed according to EPA Method 8151A.

2.2.5 Conventionals

The methods used for conventional analyses are included in Table 3.

3.0 BIOLOGICAL TESTING

Biological testing will be conducted on sediments from select sediment sampling locations. Samples submitted for biological testing will be collected synoptically with samples submitted for chemical analyses. Two sediment toxicity tests will be conducted:

- Chronic 28-day freshwater amphipod (*Hyalella azteca*)
- Chronic 20-day freshwater midge (*Chironomus tentans*).

Sediment submitted for toxicity testing will be obtained from the same homogenate as the sediment submitted for bulk chemical analyses. These sediments will be collected from the 0- to 30-cm horizon. Approximately two liters of sediment will be collected for biological effects testing. Biological testing will be performed by Northwestern Aquatic Sciences (NAS) in compliance with applicable EPA and American Society for Testing and Materials (ASTM) methods.

3.1 SAMPLE STORAGE AND SHIPPING

All samples for toxicity testing will be stored in approximately 4°C coolers until transported to NAS. Temperature within the coolers will be monitored and custody procedures will be followed throughout laboratory sample handling. Upon arrival at the laboratory NAS staff will check that containers do not contain any air space or have cracked during shipping. If these problems are observed in samples, then NAS will contact the Bioassay QA Manager, Joanna Florer, (Windward) and implement corrective action such as replacing air space with nitrogen gas to retard deterioration. All sealed containers will be stored up to 14-days at 4° C in the dark.

3.2 AMPHIPOD BIOASSAY

The purpose of this study is to characterize the chronic toxicity of freshwater sediments using 28-day exposure and the survival and growth endpoints with the amphipod *Hyalella azteca*. This protocol (Appendix A) is based on ASTM Method E 1706-95b (ASTM 2002) and EPA Method 100.1 (EPA/600/R-94/024; EPA 1994).

3.3 MIDGE BIOASSAY

The purpose of this study is to characterize the toxicity of freshwater sediments based on survival and growth of the midge *Chironomus tentans*. This protocol (Appendix B) is based on EPA Method 100.5 (EPA/600/R-99/064; EPA 2000) and ASTM Method E 1706-95b (ASTM 2002).

3.4 TOXICITY TESTING QUALITY ASSURANCE REVIEW

The bioassay project QA/QC Coordinator, Ms. Joanna Florer (Windward Environmental), will prepare a QA/QC report based on the field sampling activities and a review of the laboratory toxicity testing data. The laboratory QA/QC reports will be cited in the project QA/QC report as references. The QA/QC report will identify any field or laboratory activities that deviated from this QAPP and the referenced protocols, and will make a statement regarding the overall validity of the toxicity data collected and tested.

3.5 QA/QC BIOASSAY METHODS

Both sediment toxicity tests will incorporate standard QA/QC procedures to ensure that the test results are valid. Standard QA/QC procedures include the use of negative and positive controls and a daily measurement of the water quality in the overlying water of one replicate beaker from each treatment during testing.

The negative control will be a test using a clean, inert material and the same diluent water used in testing sediment toxicity.

The positive control will involve use of a reference toxicant to establish the relative sensitivity of the test organism. The positive control for sediment tests is typically conducted with diluent water and without sediment. Reference toxicants such as cadmium chloride, copper sulfate, and sodium dodecyl sulfate are often used in positive controls.

Bioassays require that proper water quality conditions be maintained to ensure survival of the organisms and to ensure that undue stress unrelated to test sediments is not exerted on the organisms. Parameters such as dissolved oxygen (DO), pH, ammonia, total sulfides, and temperature will be regularly measured during testing. Pore water ammonia and sulfide measurements will also be made in sacrificial test chambers at test beginning and end. Calibration information for instruments used to conduct the water quality measurements will be described in the final laboratory report.

3.6 BIOASSAY LABORATORY REPORTING

NAS will be responsible for internal checks on data reporting, and will correct errors identified during their internal quality assurance review. NAS will report results that are supported by all information recommended by ASTM protocols for quality assurance review, including:

- Cover letter discussing analytical problems (if any) and procedures

- Test methods used for bioassay testing and statistical analyses
- Source (including collection location, age, and pre-test observations) for all test organisms
- Results for survival, growth, reburial, abnormalities, water quality parameters, reference toxicants, and statistical analyses
- Results of the reference toxicant test with historical results (e.g., control charts)
- Original data sheets for water quality, survival, growth, reburial, abnormalities, reference toxicant, and statistics
- Original quality control checklists
- Custody records
- Computer diskette containing electronic files of all data.

Close contact with the laboratory will be maintained to resolve any quality control problems in a timely manner.

Guidelines for electronic data deliverables for bioassay data are as follows:

- The file should have a header with the laboratory name, test medium, and test type as described by scientific name of the organism used, life history stage or organism size range, duration of test, endpoint and unit for endpoint, and an indication of static or flow-through conditions.
- Each row should contain the following information at a minimum: sample identifier, the laboratory sample identifier (if used), replicate number, test series number, dilution (positive control only), and the result with units clearly identified.
- Positive and negative controls should be included in the file with replicates and series number if appropriate.

4.0 REFERENCES

- ASTM. 2002. Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates. ASTM Standard Method No. E 1706-00. In: *2002 Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides*. ASTM International, West Conshohocken, PA.
- EPA. 1994. Test Method 100.1, *Hyalella azteca* 10-d Survival Test for Sediments. pp. 44-50. In: *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*. EPA/600/R-94/024. U.S. Environmental Protection Agency, Office of Research and Development, Washington DC.
- EPA. 1998. Test methods for evaluating solid wastes – physical/chemical methods, SW-846. Revised methods. Update III. U.S. Environmental Protection Agency, Washington, DC.
- EPA. 2000. Test Method 100.5, Life-cycle Test for Measuring the Effects of Sediment-associated Contaminants on *Chironomus tentans*. pp. 84-91. In: *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*. Second edition. EPA/600/R-94/024. U.S. Environmental Protection Agency, National Service Center for Environmental Publications, Cincinnati, OH.
- EPA. 2002a. Columbia River Basin Fish Contaminant Survey 1996 – 1998. U.S. Environmental Protection Agency, Region 10, Seattle, WA.
- EPA. 2002b. National Recommended Water Quality Criteria: 2002. EPA-822-R-02-047. U.S. Environmental Protection Agency, Office of Water, Washington D.C.
- SEA. 2002. Round 1 Quality Assurance Project Plan, Portland Harbor RI/FS. Final Report. Prepared for Lower Willamette Group, Portland, OR. Striplin Environmental Associates, Olympia, WA.

Table 1. Water Sample Containers, Preservation, Holding Times, and Sample Volume.¹

	Containers		Preservation	Holding Time	Sample Size
	Type	Size			
Total Suspended Solids	HDPE	1 liter	4±2°C	7 days	1 liter
Total Dissolved Solids	HDPE	500 ml	4±2°C	7 days	250 ml
Total Organic Carbon	HDPE ²	250 ml	HCl or H ₂ SO ₄ to pH <2 & 4±2°C	28 days	50 ml
Metals + Mercury	HDPE ²	1 liter	5 ml of 1:1 & HNO ₃ & 4±2°C	6 months/60 days ³	100 ml
SVOCs	AG ⁴	1 liter	4±2°C	7 /40 days ⁵	1 liter
Pesticides/PCBs	AG ⁴	1 liter	4±2°C	7 /40 days ⁵	3 - 6 liters ⁶
Herbicides	AG ⁴	1 liter	4±2°C	7 /40 days ⁵	1 liter

HDPE = High Density Polyethylene

AG = Amber Glass

¹All samples will need a minimum of 5% QA. Collection of 3 times the normal volume will be necessary.

²Container is sent by lab with preservative in it.

³Based on CRITFC study (EPA 2002a) and EPA Method 1631 revision D.

⁴Organic free with Teflon lined lids, with certificate of analysis.

⁵Holding time is 7 days to extraction and extracts must be analyzed within 40 days from extraction.

⁶Six liters will be required if the laboratory does not use large volume injection (LVI) for PCBs analysis. Sample size may be reduced to 3-4 liters with LVI.

Table 2. Project Specific Method Reporting Limits, Analytical Concentration Goals, and Methods for Water Analyses.

Analytes	Analytical Method	MRL¹	ACG²	CAS#
CONVENTIONALS				
Total Suspended Solids	EPA 160.2/SM 2540D	1.0	NE ³	NA
Total Dissolved Solids	EPA 160.1/SM 2540C	10	NE	NA
Total Organic Carbon	ASTM D4129-82m 415.1	1.5	NE	NA
METALS				
		mg/L (ppm)	mg/L (ppm)	
Aluminum	EPA Method 6010B ICP	0.050	36	7782-49-2
Antimony	EPA Method 6020 ICPMS	0.0002	0.015	7429-90-5
Arsenic	EPA Method 6020 ICPMS	0.0005	0.000045	7440-66-6
Cadmium	EPA Method 6020 ICPMS Mod	** 0.0001	0.00011	7440-43-9
Chromium	EPA Method 6010B ICP	0.005	0.027	7440-47-3
Copper	EPA Method 6010B ICP	0.002	0.0032	7440-50-8
Lead	EPA Method 6020 ICPMS Mod	** 0.0005	0.00066	7439-97-6
Mercury	EPA Method 7040A CVAA	0.0001	0.001	7440-50-8
Nickel	EPA Method 6010B ICP	0.01	0.018	7439-92-1
Selenium	EPA Method 6020 ICPMS	0.0005	0.005	7440-36-0
Silver	EPA Method 6020 ICPMS	0.0005	0.0004	7782-49-2
Zinc	EPA Method 6010B ICP	0.006	0.042	7440-66-6
Hardness (Ca, Mg)	EPA Method 6010B ICP	5.0	NE	7440-70-2, 7439-95-4
PCBs Aroclors				
		µg/L (ppb)	µg/L (ppb)	
Aroclor 1016	SW846-8082	0.033	0.960	12674-11-2
Aroclor 1221	SW846-8082	0.067	0.034	11104-28-2
Aroclor 1232	SW846-8082	0.033	0.034	11141-16-5
Aroclor 1242	SW846-8082	0.033	0.034	53469-21-9
Aroclor 1248	SW846-8082	0.033	0.034	12672-29-6
Aroclor 1254	SW846-8082	0.033	0.034	11097-69-1
Aroclor 1260	SW846-8082	0.033	0.034	11096-82-5
Aroclor 1262	SW846-8082	0.033	NE	37324-23-5
Aroclor 1268	SW846-8082	0.033	NE	11100-14-4
CHLORINATED HERBICIDES				
		µg/L (ppb)	µg/L (ppb)	
Dalapon	SW846-8151A	2	1100	75-99-0
Dicamba	SW846-8151A	0.5	1100	1918-00-9
MCPA	SW846-8151A	500	18	94-74-6
Dichlorprop	SW846-8151A	1	NE	120-36-5
2,4-D	SW846-8151A	1	360	94-75-7
2,4,5-TP (Silvex)	SW846-8151A	0.25	290	93-72-1
2,4,5-T	SW846-8151A	0.25	360	93-76-5
2,4-DB	SW846-8151A	5	290	94-82-6
Dinoseb	SW846-8151A	1	36	88-85-7
MCPP	SW846-8151A	NE	36	93-65-2
ORGANOCHLORINE PESTICIDES⁴				
		µg/L (ppb)	µg/L (ppb)	
α - BHC	SW846-8081A (Manchester)	0.001	0.011	319-84-6
β - BHC	SW846-8081A (Manchester)	0.001	0.037	319-85-7
γ - BHC (Lindane)	SW846-8081A (Manchester)	0.001	0.052	58-89-9
δ - BHC	SW846-8081A (Manchester)	0.001	NE	319-86-8
Heptachlor	SW846-8081A (Manchester)	0.001	0.0038	76-44-8
Aldrin	SW846-8081A (Manchester)	0.001	0.004	309-00-2
Heptachlor epoxide	SW846-8081A (Manchester)	0.001	0.0038	1024-57-3
γ - Chlordane	SW846-8081A (Manchester)	0.001	NE	5103-74-2
α - Chlordane	SW846-8081A (Manchester)	0.001	NE	5103-71-9
Endosulfan I	SW846-8081A (Manchester)	0.001	0.056	959-98-8

Table 2. Project Specific Method Reporting Limits, Analytical Concentration Goals, and Methods for Water Analyses.

Analytes	Analytical Method	MRL ¹	ACG ²	CAS#
4,4'-DDE	SW846-8081A (Manchester)	0.002	0.200	72-55-9
Dieldrin	SW846-8081A (Manchester)	0.002	0.004	60-57-1
Endrin	SW846-8081A (Manchester)	0.002	0.036	72-20-8
Endosulfan II	SW846-8081A (Manchester)	0.002	0.056	33213-65-9
4,4'-DDD	SW846-8081A (Manchester)	0.002	0.280	72-54-8
Endrin aldehyde	SW846-8081A (Manchester)	0.002	NE	7421-93-4
4,4'-DDT	SW846-8081A (Manchester)	0.002	0.001	50-29-3
Endosulfan sulfate	SW846-8081A (Manchester)	0.002	NE	1031-07-8
Endrin ketone	SW846-8081A (Manchester)	0.002	NE	53494-70-5
Methoxychlor	SW846-8081A (Manchester)	0.015	0.030	72-43-5
Hexachlorobenzene	SW846-8081A (Manchester)	0.002	0.042	118-74-1
Toxaphene	SW846-8081A (Manchester)	0.1	0.0002	8001-35-2
Hexachlorobutadiene	SW846-8081A (Manchester)	0.002	0.860	87-68-3
oxy chlordane	SW846-8081A (Manchester)	* 0.002	NE	26880-48-8
<i>cis</i> - nonachlor	SW846-8081A (Manchester)	* 0.002	NE	5103-73-1
<i>trans</i> - nonachlor	SW846-8081A (Manchester)	* 0.002	NE	39765-80-5
2,4'-DDD	SW846-8081A (Manchester)	* 0.002	0.280	53-19-0
2,4'-DDE	SW846-8081A (Manchester)	* 0.002	0.200	3424-82-6
2,4'-DDT	SW846-8081A (Manchester)	* 0.002	0.200	789-02-6
SEMIVOLATILE ORGANIC COMPOUNDS		µg/L (ppb)	µg/L (ppb)	
1,2,4-Trichlorobenzene	SW846-8270C	1.0	190	120-82-1
1,2-Dichlorobenzene	SW846-8270C	1.0	370	95-50-1
1,3-Dichlorobenzene	SW846-8270C	1.0	6	541-73-1
1,4-Dichlorobenzene	SW846-8270C	1.0	0.500	106-46-7
2,2'-oxybis(1-chloropropane)	SW846-8270C	1.0	0.270	108-60-1
2,4-Dinitrotoluene	SW846-8270C	5.0	73	121-14-2
2,6-Dinitrotoluene	SW846-8270C	5.0	36	606-20-2
2-Chloronaphthalene	SW846-8270C	1.0	490	91-58-7
2-Nitroaniline	SW846-8270C	5.0	1.000	88-74-4
3,3'-Dichlorbenzidine	SW846-8270C SIM-LVI	* 0.0075	0.150	91-94-1
3-Nitroaniline	SW846-8270C	6.0	NE	99-09-2
4-bromophenyl-phenyl ether	SW846-8270C	1.0	NE	101-55-3
4-Chloroaniline	SW846-8270C	3.0	150	106-47-8
4-Chlorophenyl-phenyl ether	SW846-8270C	1.0	NE	7005-72-3
4-Nitroaniline	SW846-8270C	5.0	NE	100-01-6
Aniline	SW846-8270C	1.0	12	62-53-3
Benzoic Acid	SW846-8270C	60	150000	65-85-0
Benzyl Alcohol	SW846-8270C	5.0	11000	100-51-6
Bis-(2-chloroethoxy) methane	SW846-8270C	1.0	NE	111-91-1
Bis-(2-chloroethyl) ether	SW846-8270C SIM-LVI	* 0.0030	0.010	111-44-4
Hexachlorobenzene	SW846-8270C SIM-LVI	* 0.0015	0.042	118-74-1
Hexachlorobutadiene	SW846-8270C SIM-LVI	* 0.0015	0.860	87-68-3
Hexachlorocyclopentadiene	SW846-8270C	5.0	220	77-47-4
Hexachloroethane	SW846-8270C SIM-LVI	* 0.0015	4.800	67-72-1
Isophorone	SW846-8270C	1.0	71	78-59-1
Nitrobenzene	SW846-8270C	1.0	3.400	98-95-3
N-Nitrosodimethylamine	SW846-8270C SIM-LVI	* 0.0075	0.001	62-75-9
N-Nitroso-di-n-propylamine	SW846-8270C	2.0	0.010	621-64-7
N-Nitrosodiphenylamine	SW846-8270C	1.0	14	86-30-6
PAHs		µg/L (ppb)	µg/L (ppb)	
2-Methylnaphthalene	SW846-8270C SIM-LVI	0.0015	NE	91-57-6

Table 2. Project Specific Method Reporting Limits, Analytical Concentration Goals, and Methods for Water Analyses.

Analytes	Analytical Method	MRL ¹	ACG ²	CAS#
Acenaphthene	SW846-8270C SIM-LVI	0.0015	370	83-32-9
Acenaphthylene	SW846-8270C SIM-LVI	0.0015	NE	208-96-8
Anthracene	SW846-8270C SIM-LVI	0.0015	1800	120-12-7
Benzo(a)anthracene	SW846-8270C SIM-LVI	0.0015	0.092	56-55-3
Benzo(a)pyrene	SW846-8270C SIM-LVI	0.0015	0.009	50-32-8
Benzo(b)fluoranthene	SW846-8270C SIM-LVI	0.0015	0.092	205-99-2
Benzo(ghi)perylene	SW846-8270C SIM-LVI	0.0015	NE	191-24-2
Benzo(k)fluoranthene	SW846-8270C SIM-LVI	0.0015	0.920	207-08-9
Carbazole	SW846-8270C SIM-LVI	NE	3.400	86-74-8
Chrysene	SW846-8270C SIM-LVI	0.0015	9.200	218-01-9
Dibenz(a,h)anthracene	SW846-8270C SIM-LVI	0.0015	0.009	53-70-3
Dibenzofuran	SW846-8270C SIM-LVI	0.0015	24	132-64-9
Fluoranthene	SW846-8270C SIM-LVI	0.0015	1500	206-44-0
Fluorene	SW846-8270C SIM-LVI	0.0015	240	86-73-7
Indeno(1,2,3-cd)pyrene	SW846-8270C SIM-LVI	0.0015	0.092	193-39-5
Naphthalene	SW846-8270C SIM-LVI	0.0015	6.200	91-20-3
Phenanthrene	SW846-8270C SIM-LVI	0.0015	NE	85-01-8
Pyrene	SW846-8270C SIM-LVI	0.0015	180	129-00-0
Phenols			µg/L (ppb)	
2,3,4,6-Tetrachlorophenol	SW846-8270C	* 5.0	1100	58-90-2
2,4,5-Trichlorophenol	SW846-8270C	5.0	3600	95-95-4
2,4,6-trichlorophenol	SW846-8270C	5.0	3.600	88-06-2
2,4-Dichlorophenol	SW846-8270C	3.0	110	120-83-2
2,4-Dimethylphenol	SW846-8270C	3.0	730	105-67-9
2,4-Dinitrophenol	SW846-8270C	25	73	51-28-5
2-Chlorophenol	SW846-8270C	1.0	30	95-57-8
2-Methylphenol	SW846-8270C	2.0	1800	95-48-7
2-Nitrophenol	SW846-8270C	5.0	NE	88-75-5
4,6-Dinitro-2-methylphenol	SW846-8270C	15	NE	534-52-1
4-Chloro-3-methylphenol	SW846-8270C	2.0	NE	59-50-7
4-Methylphenol	SW846-8270C	1.0	180	106-44-5
4-Nitrophenol	SW846-8270C	5.0	NE	100-02-7
Pentachlorophenol	SW846-8270C SIM-LVI	0.0075	0.560	87-86-5
Phenol	SW846-8270C	2.0	22000	108-95-2
Tetrachlorophenol	SW846-8270C	* 5.0	1100	25167-83-3

Table 2. Project Specific Method Reporting Limits, Analytical Concentration Goals, and Methods for Water Analyses.

Analytes	Analytical Method	MRL¹	ACG²	CAS#
Phthalate esters		µg/L (ppb)	µg/L (ppb)	
bis(2-Ethylhexyl) phthalate	SW846-8270C	1.0	4.8	117-81-7
Butylbenzylphthalate	SW846-8270C	1.0	7300	85-68-7
Diethylphthalate	SW846-8270C	1.0	29000	84-66-2
Dimethylphthalate	SW846-8270C	1.0	360000	131-11-3
Di-n-butylphthalate	SW846-8270C	1.0	3600	84-74-2
Di-n-octylphthalate	SW846-8270C	1.0	1500	117-84-0

¹MRL are project specific and based on "clean samples".

²ACG are the lower of the EPA Region 9 Preliminary Remediation Goals (PRG) or freshwater aquatic life criteria (EPA 2002b). Hardness dependent criteria based on assumed hardness of 30 mg/L (CaCO₃).

³Not Established.

⁴Sample volume of 3000 ml to 1 ml.

*Probable reporting limit. Laboratory needs to perform MDL study.

** Reporting limit may not be achievable, considering interferences from concentratin solids in the sample and background interference (normal lab contaminant)

Table 3. Sample Analysis Methods for Water Samples.

	Analysis Method
Semivolatile Organic Compounds (SVOC)	SW846-8270C Semivolatile Organic Compounds By Gas Chromatography/Mass Spectrometry (GC/MS) SW846-8270C SIM-HVI Semivolatile Organic Compounds By GC-MS Selected Ion Monitoring with Large Volume Injection.
PCBs Aroclors®	SW846-8082 Polychlorinated Biphenyls (PCBs) By Gas Chromatography
Pesticides	SW846-8081A - Organochlorine Pesticides By Gas Chromatography, Manchester Extraction
Herbicides	SW846-8151A Chlorinated Herbicides By GC Using Methylation Or Pentafluorobenzylation Derivatization
Mercury	Method 7470A Mercury in liquid waste (Manual Cold-Vapor Technique)
Metals	SW846-6020 Inductively Coupled Plasma Mass Spectroscopy, modified for cadmium and lead SW846-6010B Inductively Coupled Plasma Atomic Emission Spectroscopy
Total Organic Carbon	ASTM D4129-82m 415.1
Total Solids	EPA-160.3/SM 2540B
Total Dissolved Solids	EPA-160.1/SM 2540C
Total Suspended Solids	EPA-160.2/SM 2540D

Table 4. QA/QC Sample Control Limits for each Analytical Group.

Analysis ^{1,2}	Analytes	Percent Recovery		RPD	PARCC % Complete	
		Sample Matrix Spike	Method Blank/LCS			
<i>Semivolatiles SW846-8270C surrogates</i>	<i>d4-2-Chlorophenol</i>	51-110	51-109	30	90	
	<i>d4-1,2-Dichlorobenzene</i>	39-92	39-91			
	<i>2,4,6-Tribromophenol</i>	34-142	40-125			
	<i>2-Fluorophenol</i>	47-109	41-109			
	<i>d5-Phenol</i>	43-112	49-105			
	<i>d5-Nitrobenzene</i>	49-118	49-114			
	<i>2-Fluorobiphenyl</i>	53-106	46-109			
	<i>d14-p-Terphenyl</i>	45-126	5-126			
	<i>Semivolatiles SW846-8270C spikes</i>	<i>Phenol</i>	47-129			59-119
		<i>2-Chlorophenol</i>	49-131			63-120
		<i>1,4-Dichlorobenzene</i>	20-88			27-85
		<i>N-nitroso-di-n-propylamine</i>	38-112			44-111
		<i>1,2,4-Trichlorobenzene</i>	20-85			26-83
		<i>4-Chloro-3-methylphenol</i>	48-113			50-101
<i>Acenaphthene</i>		42-110	51-108			
<i>4-Nitrophenol</i>		42-155	39-130			
<i>2,4-Dinitrotoluene</i>		39-121	44-118			
<i>Pentachlorophenol</i>		26-175	34-144			
<i>SIM surrogate</i>	<i>Pyrene</i>	43-110	37-126			
	<i>d10-2-Methylnaphthalene</i>	34-120	48-107			
	<i>SIM spikes</i>	<i>d14-Dibenzo (a,h) anthracene</i>	14-141	37-128		
		<i>Phenanthrene</i>	43-119	49-118		
<i>Pesticides SW846-8081A surrogates</i>	<i>Benzo (k) fluoranthene</i>	38-131	42-134	30	90	
	<i>Chrysene</i>	40-131	46-128			
	<i>Tetrachloro-meta-xylene (TCMX)</i>	38-108	47-86			
	<i>Decachlorobiphenyl</i>	30-115	68-102			
	<i>spikes</i>	<i>Lindane</i>	33-120			38-106
		<i>Heptachlor</i>	50-104			51-94
		<i>Aldrin</i>	42-102			50-100
		<i>Dieldrin</i>	43-125			68-115
<i>Endrin</i>	56-123	67-125				
<i>DDT</i>	46-126	61-105				

Table 4. QA/QC Sample Control Limits for each Analytical Group.

Analysis ^{1,2}	Analytes	Percent Recovery		RPD	PARCC % Complete
		Sample Matrix Spike	Method Blank/LCS		
PCBs SW846-8082A surrogates spikes	Tetrachloro-meta-xylene (TCMX)	36-104	49-98	30	90
	Decachlorobiphenyl	26-131	28-128		
	Aroclor 1242	47-115	58-114		
Herbicides SW846-8151 surrogates spikes	2,4-Dichlorophenylacetic acid	49-132	45-128	30	90
	2,4,5-TP	58-112	52-121		
	2,4-D	36-97	38-97		
	Dicamba	53-125	34-139		
Mercury SW846-7471A	Hg	75-125	75-125	35	90
Metals SW846-EPA 6010B , 6020	Ag, Al, As, Ca, Cd, Cr, Cu, Mg, Hg, Ni, Pb, Sb, Se, Zn	75-125	75-125	35	90
Total Organic Carbon ASTM D4129-82m 415.1	TOC	75-125	80-120	35	90
Total Dissolved Solids EPA 160.1/SM 2540C	TDS	na	na	30	90
Total Suspended Solids 160.2/SM 2540D	TSS	na	na	30	90

¹ Complete method references are provided in Table 3.

² Spikes include LCS/LCSD and MS/MSD

APPENDIX A
TOXICITY TEST PROTOCOLS:
HYALLELA AZTECA

DO NOT QUOTE OR CITE

This document is currently under review by US EPA and its federal, state, and tribal partners,
and is subject to change in whole or part.

TEST PROTOCOL

FRESHWATER AMPHIPOD, *HYALELLA AZTECA*, 28-DAY SEDIMENT SURVIVAL AND GROWTH TEST

1. INTRODUCTION

1.1 Purpose of Study: The purpose of this study is to characterize the chronic toxicity of freshwater sediments using a 28-day exposure and survival and growth endpoints with the amphipod, *Hyaella azteca*.

1.2 Referenced Method: This protocol is based on ASTM Method E 1706-95b (ASTM 1999) and EPA Method 100.1 (EPA/600/R-94/024).

1.3 Summary of Method: A summary of test conditions for the amphipod 28-day sediment survival and growth test is tabulated below. The test with *Hyaella azteca* is conducted at $23 \pm 1^\circ\text{C}$ with a 16L:8D photoperiod at an illuminance of about 50 to 100 foot candles. Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten 7-14 day old amphipods are used in each replicate. The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing. Amphipods in each test chamber are fed 1.5 mL of a YCT food daily. Each chamber receives two volume additions per day of overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. Test endpoints include survival and growth.

1. Test type	whole sediment toxicity test with renewal of overlying water
2. Test duration	28 days
3. Temperature	$23 \pm 1^\circ\text{C}$
4. Light quality	daylight fluorescent light
5. Illuminance	50-100 footcandles
6. Photoperiod	16L:8D
7. Test chamber size	300-mL high-form lipless beakers, (Pyrex® 1040 or equivalent)
8. Sediment volume	100 mL
9. Overlying water volume	175 mL
10. Renewal overlying water	2 volume additions/day (continuous or intermittent)
11. Age of test organisms	7-14 days old at test initiation
12. Organisms per test chamber	10
13. Replicates per treatment	8 recommended for routine testing (depends on design)
14. Organisms per treatment	80
15. Feeding regime	YCT food, fed 1.5 mL daily/chamber
16. Cleaning	if screens are used, clean as needed
17. Aeration	None, unless DO falls below 40% saturation
18. Overlying (test) water	Culture water, well water, surface water, site water or reconstituted water
19. Water quality	Hardness, alkalinity, conductivity, pH, ammonia-N beginning and end; temperature and dissolved oxygen daily
20. Endpoints	Survival & growth (based on weight)
21. Test acceptability criteria	Minimum control survival of 80%
22. Sample holding	14 days at 4°C in the dark (recommended)
23. Sample volume required	1L (800 mL per sediment)
24. Reference toxicant	Concurrent testing required

2. STUDY MANAGEMENT

2.1 Sponsor's Name and Address:

2.2 Sponsor's Study Monitor:

2.3 Name of Testing Laboratory:

Northwestern Aquatic Sciences
3814 Yaquina Bay Road, P.O. Box 1437
Newport, OR 97365.

2.4 Test Location:

2.5 Laboratory's Personnel to be Assigned to the Study:

Study Director: _____
Quality Assurance Unit: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 Proposed Testing Schedule: Tests are normally begun within 14 days of sample collection. Reference toxicant test to be run concurrently.

2.7 Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. TEST MATERIAL

The test materials are freshwater sediments. The control, reference, and test sediments are placed in solvent cleaned 1 L glass jars fitted with PTFE-lined screw caps. At the laboratory the samples are stored at 4°C in the dark. The original sealed containers may normally be stored for up to 14 days prior to testing. If jars are not full when received or if sediment is removed for testing, headspaces should be filled with nitrogen to retard deterioration. A negative control sediment is collected from a clean site. In addition, a reference sediment, a clean sediment with physical characteristics similar to the test sediments, may be employed as a comparison station.

4. TEST WATER

Test water (overlying water) at NAS is normally moderately hard synthetic water at a hardness of 80-100 mg/L as CaCO₃ and alkalinity of 60-70 mg/L as CaCO₃. Dilution water is prepared from Milli-Q reagent grade water and reagent grade chemicals. Test water may also be well water, surface water or site water, depending on the study design.

5. TEST ORGANISMS

5.1 Species: amphipod, *Hyalella azteca*.

5.2 Source: Cultured at NAS. Originally purchased from ESE in Gainesville, FL. Alternatively animals may be purchased from a commercial supplier.

5.3 Age: 7-14 days old at start of test; with the growth endpoint, it may be desirable to employ animals at the younger end of the age range.

5.3 Acclimation and Pretest Observation: Cultures are maintained at $23 \pm 1^\circ\text{C}$ under a 16:8 L:D photoperiod. Cultured amphipods are fed dried maple leaves with occasional Tetramin® flake or rabbit chow supplements. Acclimation of test organisms to the test water may be desirable, depending on culture water, but it is not required by ASTM. If test organisms are to be acclimated, fifty percent of the holding water is changed daily with the addition of test water.

6. DESCRIPTION OF TEST SYSTEM

6.1 Test Chambers and Environmental Control: Test chambers used in the toxicity test are 300-mL high-form lipless glass beakers. Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or by placement in a temperature-controlled room. Aeration is not employed unless dissolved oxygen drops below 40% saturation. The test is conducted under an illuminance of 50-100 footcandles with a 16L:8D photoperiod.

6.2 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: The test involves exposure of amphipods to test, control, and reference sediments. The sediments are placed on the bottom of the test containers and are overlain with test water. The test exposure is for 28 days. The renewal of overlying water consists of two volume additions per day, either continuous or intermittent. Each treatment consists of eight replicate test containers, each containing 10 organisms. Test chamber positions are completely randomized. Test organisms are randomly distributed to the test chambers. Blind testing is normally used.

7.2 Setup of Test Containers: Sediments are homogenized and placed in test chambers on the day before addition of test organisms. Sediment (100 ml) is placed into each of eight replicate beakers. After addition of the sediment, 175 ml of test water is gently added to each beaker in a manner to prevent resuspension. The overlying water is replaced twice daily. The test begins when amphipods are introduced to the test chambers. Initial water quality measurements are taken prior to the addition of test organisms.

7.3 Effect Criterion: The acute effect criteria used in the 28-day amphipod bioassay are mortality and growth. Death is defined as the lack of movement of body or appendages on response to tactile stimulation. Growth is measured as change in dry weight.

7.4 Test Conditions: No aeration is employed unless dissolved oxygen falls below 40% saturation. The test temperature employed is $23 \pm 1^\circ\text{C}$. A 16:8, L:D photoperiod is used. Illumination is supplied by daylight fluorescent lamps at 50-100 footcandles. The overlying water is replaced twice daily.

7.5 Beginning the Test: On the day the test begins, amphipods are impartially counted into small containers of test water (10/container). The test is begun by rinsing test organisms into the equilibrated test containers. For the growth endpoint, time-zero weight data should be collected.

7.6 Feeding: Amphipods are fed 1.5 mL of YCT daily per test chamber. A feeding may be skipped if there is a build up of excess food. However, all beakers must be treated similarly.

7.7 Test Duration, Type and Frequency of Observations, and Methods: The duration of the acute toxicity test is 28 days. The type and frequency of observations to be made are summarized as follows:

TYPE OF OBSERVATION	TIMES OF OBSERVATION (ASTM 1999)
<i>BIOLOGICAL DATA</i>	
Survival, growth	Day 28
<i>PHYSICAL AND CHEMICAL DATA</i>	
Hardness, alkalinity, ammonia-N, conductivity, pH, dissolved oxygen, and temperature	Beginning and end of test in overlying water of one replicate beaker from each treatment.
Dissolved oxygen, temperature	Daily in overlying water of one replicate beaker from each treatment.
Optional pore water ammonia and/or sulfide	In test sediments prior to initiating the tests. Optionally in sediments from sacrificial test chambers at test beginning and/or end.

Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH is measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Temperature is measured with a calibrated mercury thermometer or telethermometer. Conductivity is measured with a conductivity meter. Hardness and alkalinity are measured using titrimetric methods. Total soluble sulfide and total ammonia-N were measured using Hach test kits based on the methylene blue (EPA Method 376.2) and salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric methods, respectively; samples were not distilled prior to analysis.

Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessary to pool water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water.

7.8 Growth Measurement: Growth is measured as average dry weight of animals in a test replicate at the end of the test on day 28. Pooled animals from each test replicate are gently blotted and placed into tared aluminum weigh pans. The pans are dried at 60-90°C to constant weight. The dried amphipods are placed into a dessicator and weighed as soon as possible to the nearest 0.01 mg (desirable to use 0.001 mg). The total weight of the dried amphipods in each pan is divided by the number of amphipods weighed to obtain an average dry weight per surviving amphipod per replicate.

7.9 Criteria of Test Acceptance: The test results are acceptable if the minimum survival of organisms in the control treatment at the end of the test is at least 80%.

8. DATA ANALYSIS

The endpoints of the toxicity test are survival and growth. Survival is obtained as a direct count of living organisms in each test container at the end of the test. Average amphipod dry weight, also measured at the end of the test, may be used to compare growth between treatment sediments and the control or reference sediment. Ordinarily the following data analysis is performed. Due to special requirements, alternative methods may be used. The means and standard deviations are calculated for each treatment level. Identification of toxic sediments is established by statistical comparison of test endpoints between test and control or reference sediments. Between treatment comparisons may be made using a Student's t-test or Wilcoxon's Two-Sample test, where each treatment is compared to the control or the reference sediment. An arcsine-square root transformation of proportional data, and tests for normality and heterogeneity of variances, are performed prior to statistical comparisons.

9. REPORTING

The final report of the test results must include all of the following standard information at a minimum: name and identification of the test; the investigator and laboratory; date and time of test beginning and end; information on the test material; information on the source and quality of the overlying/test water; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including feeding, if any, and water quality; definition of the effect criteria and other observations; responses, if any, in the control treatment; tabulation and statistical analysis of measured responses and a summary table of endpoints; a description of the statistical methods used; any unusual information about the test or deviations from procedures; reference toxicant testing information.

10. STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change, the reason for the change, the date the change took effect and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

11. REFERENCE TOXICANT

The reference toxicant test is a standard multi-concentration toxicity test using a specified chemical toxicant to evaluate the performance of test organisms used in the study. Reference toxicant tests are 96-hour, water only exposures, not 28-day sediment exposures. The reference toxicant test is run concurrently. Performance is evaluated by comparing the results of the reference toxicant test with historical results (e.g., control charts) obtained at the laboratory.

12. REFERENCED GUIDELINES

ASTM. 1999. Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates. ASTM Standard Method No. E 1706-95b. Am. Soc. Test. Mat., Philadelphia, PA.

U.S. EPA. 1994. Section 11, Test Method 100.1, Hyalella azteca 10-d Survival Test for Sediments, pp. 44-50 In: Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. EPA/600/R-94/024.

Weber, C.I. (Ed.) 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). EPA/600/4-90/027F.

13. APPROVALS

_____ for _____
Name Date

_____ for Northwestern Aquatic Sciences
Name Date

APPENDIX B
TOXICITY TEST PROTOCOLS:
CHIRONOMUS TENTANS

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This document is currently under review by US EPA and its federal, state, and tribal partners,
and is subject to change in whole or part.

TEST PROTOCOL

FRESHWATER MIDGE, *CHIRONOMUS TENTANS*, 20-DAY SEDIMENT TOXICITY TEST

1. **INTRODUCTION**

1.1 **Purpose of Study:** The purpose of this study is to characterize the toxicity of freshwater sediments based on midge survival and growth using the midge, *Chironomus tentans*.

1.2 **Referenced Method:** This protocol is based on EPA Method 100.5 (EPA/600/R-99/064) and ASTM Method E 1706-95b (ASTM 1996).

1.3 **Summary of Method:** A summary of test conditions for the midge 20-day sediment toxicity test is tabulated below. The 20-day sediment toxicity test with *Chironomus tentans* is conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 50-100 footcandles. Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten <24 hour-old (first-instar) midge larvae are used in each replicate. The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing. Midges in each test chamber are fed 1.5 mL (contains 6.0 mg of dry solids) of fish food flakes suspension daily. Each chamber receives two volume additions per day of overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. Test endpoints include survival and growth (dry weight or ash-free dry weight (AFDW)).

1. Test type	whole sediment toxicity test with renewal of overlying water
2. Test duration	20 days
3. Temperature	23 ± 1°C
4. Light quality	daylight fluorescent light
5. Illuminance	50-100 footcandles
6. Photoperiod	16L:8D
7. Test chamber size	300-mL high-form lipless beakers (Pyrex® 1040 or equivalent)
8. Sediment volume	100 mL
9. Overlying water volume	175 mL
10. Renewal overlying water	2 volume additions/day (continuous or intermittent)
11. Age of test organisms	< 24 hour-old (first instar) larvae
12. Organisms per test chamber	10
13. Replicates per treatment	8 recommended for routine testing (depends on design)
14. Organisms per treatment	80
15. Feeding regime	Fish food flakes, fed 1.5 mL daily/chamber (1.5 mL contains 6.0 mg of dry solids)
16. Aeration	None, unless DO falls below 2.5 mg/L
17. Overlying (test) water	Culture water, well water, surface water, site water or reconstituted water
18. Water quality	Hardness, alkalinity, conductivity, pH, ammonia-N beginning and end; temperature and dissolved oxygen daily
19. Endpoints	Survival and growth (dry weight or ash-free dry weight.)
20. Test acceptability criteria	Minimum control survival of 70%; mean AFDW of surviving control organisms = 0.48 mg (or dry weight = 0.6 mg)
21. Sample holding	up to 8 weeks at 4°C in the dark (14 days when volatiles in sediments)
22. Sample volume required	1L (800 mL per sediment)
23. Reference toxicant	KCl

NAS-XXX-CT4c

October 18, 2000

2. STUDY MANAGEMENT

2.1 Sponsor's Name and Address:

2.2 Sponsor's Study Monitor:

2.3 Name of Testing Laboratory:

Northwestern Aquatic Sciences
3814 Yaquina Bay Road, P.O. Box 1437
Newport, OR 97365.

2.4 Test Location:

2.5 Laboratory's Personnel to be Assigned to the Study:

Study Director: _____
Quality Assurance Unit: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 Proposed Testing Schedule: Sediments should be tested sometime between sediment collection and 8 weeks storage. Sediments that contain high concentrations of labile chemicals such as ammonia and volatile organics should be tested as soon as possible after collection, but no later than within two weeks. A 96-hr, water-only reference toxicant test may be run concurrently, or periodic reference toxicant tests run on cultures may be used to assess organism sensitivity.

2.7 Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. TEST MATERIAL

The test materials are freshwater sediments. The control, reference, and test sediments are placed in solvent cleaned 1 L glass jars fitted with PTFE-lined screw caps. At the laboratory the samples are stored at 4°C in the dark. The original sealed containers may be stored for up to 8 weeks prior to testing, depending on the testing requirements. If jars are not full when received or if sediment is removed for testing, headspaces may be filled with nitrogen to retard deterioration, depending on testing requirements. A negative control sediment is collected from a clean site. In addition, a reference sediment, a clean sediment with physical characteristics similar to the test sediments, is normally employed as a comparison station. Test sediments should be homogenized before use in a test.

4. TEST WATER

Test water (overlying water) at NAS is normally *C. tentans* culture water, which is moderately hard synthetic water at a hardness of 80-100 mg/L as CaCO₃ and alkalinity of 60-70 mg/L as CaCO₃. Dilution water is prepared from Milli-Q reagent grade water and reagent grade chemicals. Test water may also be well water, surface water or site water depending on the study design.

5. TEST ORGANISMS

5.1 Species: midge, *Chironomus tentans*.

5.2 Source: Cultured at NAS. Originally obtained from U.S. EPA Environmental Research Lab, Duluth, MN.

5.3 Age: < 24 hour-old (first instar) larvae

5.4 Acclimation and Pretest Observation: Cultures are maintained at approximately 23°C under a 16:8 L:D photoperiod. The culture water is moderately hard synthetic water. Midges are fed *Selenastrum* algae and finely ground Tetrafin flakes in suspension (10g Tetrafin in 100 mL Milli-Q water).

6. DESCRIPTION OF TEST SYSTEM

6.1 Test Chambers and Environmental Control: Test chambers used in the toxicity test are 300-mL high-form lipless glass beakers (Pyrex® 1040 or equivalent). Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or by placement in a temperature-controlled room. Aeration is not employed unless dissolved oxygen drops below 2.5 mg/L. The test is conducted under an illuminance of 50-100 footcandles with a 16L:8D photoperiod.

6.2 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: The test involves exposure of midge larvae to test, control, and reference sediments. The sediments are placed on the bottom of the test containers and are overlain with test water. The test exposure is for 20 days. The renewal of overlying water consists of two volume additions per day, either continuous or intermittent. Each treatment consists of eight replicate test containers, each containing 10 organisms. Test chamber positions are completely randomized. Test organisms are randomly distributed to the test chambers. Blind testing is normally used.

7.2 Setup of Test Containers: Sediments are homogenized and placed in test chambers on the day before addition of test organisms. Sediment (100 ml) is placed into each of eight replicate beakers. After addition of the sediment, 175 ml of test water is gently added to each beaker in a manner to prevent resuspension. The overlying water is replaced twice daily. The test begins when midges are introduced to the test chambers. Initial water quality measurements are taken prior to the addition of test organisms.

7.3 Effect Criteria: The effect criteria used in the midge bioassay are survival (mortality) and growth. Mortality is defined as the lack of movement of body or appendages on response to tactile stimulation. Growth is determined by using either dry weight or ash-free dry weight measurements.

7.4 Test Conditions: No aeration is employed unless dissolved oxygen falls below 2.5 mg/L. The test temperature employed is 23°C (range of ± 1°C). A 16:8, L:D photoperiod is used. Illumination is supplied by daylight fluorescent lamps at 50-100 footcandles. The overlying water is replaced twice daily.

7.5 Beginning the Test: The test is begun by adding the organisms to the equilibrated test containers as previously described.

October 18, 2000

7.6 Feeding: Midge larvae are fed 1.5 mL daily per test chamber (1.5 mL contains 6.0 mg of dry solids). A feeding may be skipped if there is a build up of excess food. However, all beakers must be treated similarly.

7.7 Test Duration, Type and Frequency of Observations, and Methods: The duration of the acute toxicity test is 20 days. The type and frequency of observations to be made are summarized as follows:

TYPE OF OBSERVATION	TIMES OF OBSERVATION (ASTM 1996)
<i>BIOLOGICAL DATA</i>	
Survival, growth	Day 20
<i>PHYSICAL AND CHEMICAL DATA</i>	
Hardness, alkalinity, ammonia-N, conductivity, pH, dissolved oxygen, and temperature	Beginning and end of test in overlying water of one replicate beaker from each treatment.
Dissolved oxygen, temperature	Daily in overlying water of one replicate beaker from each treatment.

Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH is measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Temperature is measured with a calibrated mercury thermometer or telethermometer. Conductivity is measured with a conductivity meter. Hardness and alkalinity are measured using titrimetric methods. Ammonia-nitrogen is measured using the salicylate colorimetric method (Clin. Chim. Acta 14:403, 1996).

7.8 Growth Measurement: Growth can be measured as either dry weight or ash-free dry weight (AFDW) of animals in a test replicate at the end of the test on day 20. For dry weight, pooled animals from each test replicate are rinsed with deionized water, gently blotted and placed into tared aluminum weigh pans. For dry weight measurements, the pans are dried at 60-90°C to constant weight. The dried organisms are placed into a dessicator and weighed as soon as possible to the nearest 0.01 mg. The total weight of the dried midge in each pan is divided by the number of midge weighed to obtain an average dry weight per midge. For ash-free dry weights, the weigh pans are ashed before use. Then all living larvae per replicate are combined and dried to a constant weight (60°C for 24 h). Then the samples are brought to room temperature in a dessicator and weighed to the nearest 0.01 mg to obtain mean weights per surviving organism per replicate. The dried larvae in the pan are then ashed at 550°C for 2 h. The pan with the ashed larvae is then reweighed and 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.

7.9 Criteria of Test Acceptance: The test results are acceptable if the minimum survival of organisms in the control treatment at the end of the test is at least 70% and the average dry weight of *C. tentans* in the surviving controls is at least 0.6 mg (or 0.48mg/surviving organism as AFDW).

8. DATA ANALYSIS

The endpoints of the toxicity test are survival and growth. Survival is obtained as a direct count of living organisms in each test container at the end of the test. Average midge dry weight or ash-free dry weight, also measured at the end of the test, may be used to compare growth between treatment sediments and the control or reference sediment. Ordinarily the following data analysis is performed. Due to special requirements, alternative methods may be used. The means and standard deviations are calculated for each treatment level. Identification of toxic sediments is established by statistical comparison of test endpoints between test and control or reference sediments. Between treatment comparisons may be made using a Student's t-test or Wilcoxon's Two-Sample test, where each treatment is compared to the control or the reference sediment. An arcsine-square root transformation of proportional data, and tests for normality and heterogeneity of variances, are performed prior to statistical comparisons.

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9. REPORTING

The final report of the test results must include all of the following standard information at a minimum: name and identification of the test; the investigator and laboratory; date and time of test beginning and end; information on the test material; information on the source and quality of the overlying/test water; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including feeding, if any, and water quality; definition of the effect criteria and other observations; responses, if any, in the control treatment; tabulation and statistical analysis of measured responses and a summary table of endpoints; a description of the statistical methods used; any unusual information about the test or deviations from procedures; reference toxicant testing information.

10. STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change, the reason for the change, the date the change took effect and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

11. REFERENCE TOXICANT

The reference toxicant test is a standard multi-concentration toxicity test using a specified chemical toxicant to evaluate the performance of test organisms used in the study. Reference toxicant tests are 96-hour, water only exposures, not 20-day sediment exposures. The reference toxicant test is normally run concurrently; however, for this 20-day test periodic reference toxicant tests run on the cultures may be used to evaluate organism sensitivity. Performance is evaluated by comparing the results of the reference toxicant test with historical results (e.g., control charts) obtained at the laboratory.

12. REFERENCED GUIDELINES

ASTM. 1996. Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates. ASTM Standard Method No. E 1706-95b. Am. Soc. Test. Mat., Philadelphia, PA.

U.S. EPA. 2000. Test Method 100.5, Life-cycle Test for measuring the Effects of Sediment-associated Contaminants on *Chironomus tentans*, pp. 84-91. In: Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. Second edition. EPA/600/R-99/064.

Weber, C.I. (Ed.) 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). EPA/600/4-90/027F.

13. APPROVALS

_____ for _____
Name Date

_____ for Northwestern Aquatic Sciences
Name Date