

9 TIER 3 BIOLOGICAL TESTING: BIOASSAYS

Tier 3 biological testing of dredged material is required when chemical testing results indicate the potential for unacceptable adverse environmental or human health effects. Biological testing could include:

Bioassays– used to evaluate potential toxicity effects on benthic invertebrates – discussed in this chapter.

Bioaccumulation tests--used to evaluate the bioavailability of certain chemicals which are known or suspected agents affecting human or ecological health in the marine environment– discussed in Chapter 10.

The standard suite of bioassays for either marine or freshwater sediment in Tier 3 evaluations is triggered by **exceeding** one or more screening levels for chemicals of concern in the dredged material (see **Table 8-2**).

Laboratories providing biological effects data for DMMP projects must be accredited by the Department of Ecology for the methods used to produce the data. Additional information related to bioassay testing under the DMMP can be found on the [DMMO website](#).

9.1 MARINE BIOASSAYS

The suite of three bioassays used in the DMMP program includes both acute and chronic tests to characterize toxicity of whole sediment. Bioassays used for marine/estuarine evaluations are:

1. 10-day amphipod mortality test (acute toxicity)
2. 20-day juvenile infaunal growth test (chronic toxicity)
3. Sediment larval development test (acute toxicity)

The protocols for the required bioassays can be found in the Puget Sound Protocols and Guidelines ([PSEP, 1995](#)) and DMMP [SMARM updates](#). The protocols describe field collection and processing methods, bioassay specific QA/QC, and data reporting procedures. Also, general protocols are provided for field collection of surficial test sediments and for general QA/QC procedures that apply to all sediment bioassays.

9.1.1 Bioassay Species

The DMMP recommends the following listed species for marine bioassay testing. **If recommended species are not available, please contact the DMMO prior to initiating testing with a non-recommended species.**

1. 10-Day Amphipod Mortality Test

- *Eohaustorius estuarius* – most commonly used species; can be used with grain-size distributions ranging from 0 to 100% fines, as long as the clay fraction <20%; and in interstitial salinities ranging from 2 ppt to 28 ppt.
- *Ampelisca abdita* – recommended if test sediment contains greater than 20% clay and salinities of 28 ± 1 ppt

- *Rhepoxynius abronius* – alternative species for use in coarser-grained sediments (i.e. fines <60%) and salinities of 28 ± 1 ppt.
2. **20-Day Juvenile Infaunal Growth Test**
 - *Neanthes arenaceodentata* (Los Angeles karyotype)
 3. **Sediment Larval Development Test.**
 - Bivalve: *Mytilus galloprovincialis*
 - Echinoderm: *Dendraster excentricus*

9.1.2 10-day Amphipod Mortality Test

This bioassay is an acute test that measures survival of infaunal amphipods to evaluate the toxicity of sample sediments.

9.1.2.1 Amphipod Species Selection

The DMMP agencies generally recommend using *Eohaustorius estuarius*, as this species is relatively insensitive to salinity changes and effects of grain size, except for high clay (>20%) content. *Ampelisca abdita* is also relatively insensitive to the effects of grain size and is the recommended species when testing sediments with relatively high clay content (>20%). *Rhepoxynius abronius* has shown sensitivity to high percent fines in sediments, particularly high clay content sediments, and has exhibited mortalities greater than 20 percent in clean, reference area sediments (DeWitt *et al.*, 1988; Fox, 1993). It should only be selected when testing coarser sediments (<60% fines). Proposed species must be coordinated through the DMMO, and the rationale for species selection must be documented in the sampling and analysis plan for the proposed dredging project. Appropriate negative control sediment must be used for the test species selected. More information on amphipod species selection can be found in [DMMP 1999](#).

9.1.3 20-day Juvenile Infaunal Growth Test (*Neanthes*)

This bioassay is a sublethal bioassay, testing for chronic rather than acute (fatal) toxicity to the nereid worm *Neanthes arenaceodentata*. The growth of this worm is used as an indication of sublethal toxicity. Testing results should be reported on an ash-free dry-weight (AFDW) basis. The AFDW procedure eliminates weight from sediment in the gut, thereby providing a more accurate measurement of the change in biomass during the exposure period.

9.1.4 Sediment Larval Development Test

The sediment larval test uses the planktonic larval form of a benthic invertebrate to test for acute toxicity to this life stage. Larvae are introduced into chambers of test sediment and overlying water directly after fertilization. Development and survival are tracked for the 48 to 60 hours of larval growth.

9.1.4.1 Larval Species Selection

This test uses larvae of either an echinoderm or bivalve species. *Dendraster excentricus* is the recommended echinoderm species and *Mytilus galloprovincialis* is the recommended bivalve species. If both of these species are unavailable, laboratories may propose use of alternative species such as the bivalve *Crassostrea gigas*. **Use of alternative species should proceed only after DMMP coordination and approval.**

9.1.4.2 Special Considerations for Sediment Larval Bioassay

Because the larval stage is a sensitive one, care must be taken during the test to insure that non-treatment factors for larval survival and development are controlled. The PSEP Protocols should be followed carefully to insure that useable data are collected.

For the sediment larval test, adults must be collected in spawning condition or must be induced to spawn in the laboratory. Therefore, seasonality plays a role in selecting a test organism for this bioassay. Viable test organisms are most difficult to obtain in the fall and early winter and the probability of performance problems increases during that time. The DMMP agencies recommend that biological testing be avoided late in the calendar year if at all possible.

When testing dredged material with high concentrations of fines, wood waste or other flocculent material, applicants may elect to use the resuspension protocol (see [DMMP, 2013](#)) in lieu of the standard PSEP protocol termination procedure, in order to reduce false positives from normally developing larvae being entrained in the flocculent material. The decision to use the resuspension protocol should be made in coordination with the DMMP agencies for approval before use. For routine testing of sediments with lower fractions of fines, wood waste or flocculent material, the standard PSEP protocol should be used.

9.2 QUALITY ASSURANCE/QUALITY CONTROL IN MARINE BIOASSAYS

The following QA/QC guidelines apply to the standard suite of marine bioassays.

9.2.1 Negative Control and Reference Samples

For the amphipod and juvenile infaunal species biological tests, a negative control sediment is run with each test batch. The negative control sediment for the amphipod test is taken from the test organism collection site (see additional information in 9.2.2). The juvenile infaunal growth test, using laboratory-cultured *Neanthes arenaceodentata*, requires collection of negative control sediment from an appropriate area such as West Beach, Whidbey Island. For the sediment larval test, a negative seawater control is required. The negative control provides an estimate of test organism general health during the test exposure period.

In addition to the negative control, at least one reference sediment must be run with each test batch for each bioassay. The primary purpose of the reference sediment is to control for non-treatment effects due to grain size. Reference sediment is collected from one of the reference sediment collection sites in Puget Sound, Grays Harbor or Willapa Bay. The fines content (silt + clay) of the reference material should ideally fall within 10% of the fines content of the test sediments. For dredged material with relatively coarse-grained sediments (> 80% sand), the dredger can opt to rely solely on the control sediment (see guidance below on when it is appropriate to use control sediments as a reference).

9.2.2 Selection of Negative Control Sediments

An appropriate negative control sediment must be used for the amphipod mortality and *Neanthes* growth tests. All bioassays must be conducted using well-established negative (clean) controls. Such controls are clean, nontoxic seawater and/or sediment samples taken from outside each study area. *Rhepoxynius abronius* and *Eohaustorius estuarius* typically inhabit well-sorted, fine sand while *Ampelisca abdita* is a tube-dwelling amphipod found mainly in protected areas and is often abundant in sediments with a high organic content. *Ampelisca* generally inhabits sediments from

fine sand to mud and silt without shell, although it can also be found in relatively coarser sediments with a sizable fine component (PSEP, 1995).

The best way to ensure a good negative control is to collect the control sediment from the same location at which the test organisms are collected. *Neanthes arenaceodentata* is cultured in the lab rather than field-collected. However, PSEP (1995) states that, "For the *Neanthes* bioassay, sand should be used as the control sediment." West Beach of Whidbey Island is most often used as a collection site for clean control sediment. From PSEP (1995), "*Neanthes* maintained in West Beach sand exhibited low mortality and high percentage increases in biomass during the exposure period, indicating that West Beach sand is a suitable material for a control sediment."

Sediments proposed for use as negative controls must be approved before bioassays commence. If an area without a proven track record is proposed for collection of negative control sediment, sufficient data (such as grain size, organic carbon content, chemical data, bioassay results) must be submitted before its use can be approved by the regulatory agencies.

9.2.3 Use of Control Sediments as Reference Sediments

When reference sediment fails to meet its performance standard, and more than one reference has been collected, [DMMP/EMS \(1996\)](#) provides procedures for statistical comparisons. If no reference sediments meet performance standards, or if the control sediment is closer in grain size to one or more stations being evaluated than any of the remaining reference sediments, the control sediment could be considered an acceptable substitute for the reference sediment and the data interpreted accordingly.

If a control sediment is substantially dissimilar to the site stations and a failed reference sediment in its physical characteristics (e.g., >25% difference in fines), it may still be used as a substitute for the reference station if both the agencies and the project proponent agree that this is appropriate. Otherwise, the data will be considered unusable and data from the bioassay(s) in question will need to be rejected and tests possibly rerun.

9.2.4 Quality Control Limits for the Negative Control

All three bioassays have negative control performance standards (see **Table 9-1**). In the amphipod and juvenile infaunal bioassay tests, control mortality over the exposure period must be less than or equal to 10 percent. This represents a generally accepted level of mortality of test organisms under control conditions, where the bioassay (in terms of test organism health) is still considered a valid measure of effects of the test treatments. If control mortality is greater than 10 percent, the bioassay test will generally have to be repeated, although that determination must be made in consultation with the agencies through the DMMO. Additionally, for the *Neanthes* 20-day growth bioassay there is a negative control performance guideline of greater than 0.72 mg/individual/day as a target growth rate, with negative control growth rates below 0.38 mg/individual/day considered a QA/QC failure. Laboratories failing to achieve a control growth rate greater than 0.38 mg/individual/day may be required to retest. For the sediment larval test, the performance standard for the seawater negative control combined endpoint (mortality + abnormality) is 30 percent or less.

9.2.5 Quality Control Limits for the Reference Sediment

Performance guidelines for reference sediments are listed in **Table 9-1**. The mean amphipod test mortality for the reference sediment must not exceed 20 percent absolute over the mean negative

control sediment mortality. For the juvenile infaunal growth test, the reference sediment mean mortality must be less than or equal to 20 percent at the end of the exposure period, and the mean growth rate must be greater than or equal to 80 percent of the control sediment's mean growth rate. The seawater-normalized combined endpoint (mortality + abnormality) observed in the reference sediment for the sediment larval test must not exceed 35 percent. Failure to meet the reference sediment performance standard for a bioassay may require that the bioassay be rerun with a new reference sediment. If a performance guideline is not met for reference sediment, the DMMO should be contacted as soon as possible to coordinate with the agencies regarding a retest.

9.2.6 Positive Control - Reference Toxicant

An appropriate reference toxicant must be run with each batch of test sediments as a positive control to assess the test organism sensitivity. The LC₅₀ or EC₅₀ must be within the 95 percent confidence interval of responses expected for the toxicant used.

9.2.7 Water Quality Monitoring

Temperature, aqueous salinity, pH, and dissolved oxygen should be monitored on a daily basis for the amphipod and sediment larval tests, and every three days for the 20-day *Neanthes* growth test. Total sulfides and ammonia should be measured at least at test initiation and termination for all three tests (reference earlier sections discussing interferences here). Interstitial salinity should be measured prior to test initiation. The test protocols for each of these bioassays specify acceptable ranges for these parameters. Water quality data can be critical in the interpretation of bioassay results.

9.3 MARINE BIOASSAY INTERPRETIVE CRITERIA

The response of bioassay organisms exposed to the sediment sample representing each DMMU will be compared to the response of these organisms in both control and reference treatments. This comparison will determine whether the material is suitable for unconfined, open water disposal relative to the Clean Water Act (CWA) Section 404(b)(1) Guidelines (see **Table 9-1**).

The determination of an environmentally significant response involves two conditions: first, that the response in the tested DMMU must be greater than 20 percent different from the control response; and, second, that a comparison between mean test and mean reference responses be statistically significant. For the latter determination, the following guidelines are to be followed:

1. Multiple comparison tests (e.g., ANOVA, Dunnett's) are not to be used.
2. A null hypothesis shall be selected that reflects the one-tailed t-test approach and the type of endpoint being evaluated.
3. Bioassay data expressed in percent should be transformed prior to statistical testing using the arcsine –square-root transform to stabilize the variances and improve the normality of the data. *Neanthes* growth data may require a square root or log transformation.
4. Bioassay data should then be tested for normality and homogeneity of variances, using the Shapiro-Wilk test (*W* test) and Levene's test, respectively.
5. Bioassay data passing both tests should be tested for statistical difference using a one-tailed Student's t-test.

6. Data passing the *W* test but failing Levene's test should be tested for statistical difference using the approximate t-test.
7. Data failing the *W* test but passing Levene's test should be tested for statistical difference using the non-parametric Mann-Whitney test.
8. Data failing both the *W* test and Levene's test should be converted to ranks and tested with a t-test.

Seattle District has developed statistical analysis software called BioStat to facilitate bioassay statistical comparisons with appropriate reference sediments. Submittal of screen shots or statistical reports from BioStat will provide the documentation necessary to support summarized interpretations of bioassay data in the sediment characterization report.

9.3.1 One-hit failure

When **any one** biological test exhibits a test sediment response that exceeds the bioassay-specific guidelines (below) relative to the negative control and reference, and which is statistically significant in comparison to the reference, the DMMU is judged to be unsuitable for unconfined open-water disposal (see **Table 9-1**).

Amphipod Bioassay. For the amphipod bioassay, mean test mortality greater than 20 percent absolute over the mean negative control response, and greater than 10 percent (dispersive) or 30 percent (nondispersive) absolute over the mean reference sediment response, and statistically significant compared to reference ($\alpha = 0.05$), is considered a "hit" under the "single-hit" guidelines.

Juvenile Infaunal Growth Test. Juvenile infaunal growth test results that show a mean individual growth rate (AFDW) less than 80 percent of the mean negative control growth rate, and less than 70 percent (dispersive) or 50 percent (nondispersive) of the mean reference sediment growth rate, and statistically significant compared to reference ($\alpha = 0.05$), constitute a hit under the single-hit rule.

Sediment Larval Bioassay. For the sediment larval bioassay, test and reference sediment responses are normalized to the negative seawater control response. This normalization is performed by dividing the number of normal larvae from the test or reference treatment at the end of the exposure period by the number of normal larvae in the seawater control at the end of the exposure period, and multiplying by 100 to convert to percent. The normalized combined mortality and abnormality (NCMA) is then 100 minus this number. If the mean NCMA for a test sediment is greater than 20 percent, and is 15 percent (dispersive) or 30 percent (nondispersive) greater than the mean reference sediment NCMA, and statistically significant compared to reference ($\alpha = 0.10$), it is considered a hit under the single-hit rule.

9.3.2 Two-hit failure

When **any two** biological tests (amphipod, juvenile infaunal growth or sediment larval) exhibit test sediment responses which are less than the bioassay-specific reference-comparison guidelines noted above for a single-hit failure, but are statistically significant compared to the reference sediment (and less than 70 percent of the mean reference sediment growth rate for the *Neanthes* bioassay for nondispersive sites), the DMMU is judged to be unsuitable for unconfined open-water disposal.

Table 9-1. Marine Bioassay Performance Standards and Evaluation Guidelines

Bioassay	Negative Control Performance Standard	Reference Sediment Performance Standard	Dispersive Disposal Site Interpretation Guidelines		Nondispersive Disposal Site Interpretation Guidelines	
			1-hit rule	2-hit rule	1-hit rule	2-hit rule
Amphipod Mortality	$M_C \leq 10\%$	$ M_R - M_C \leq 20\%$	$ M_T - M_C > 20\%$ and M_T vs. M_R SD ($p=.05$) AND			
			$M_T - M_R > 10\%$	NOCN	$M_T - M_R > 30\%$	NOCN
Larval Development	$N_{C+I} \geq 0.70$	$N_R \div N_C \geq 0.65$	$N_T \div N_C < 0.80$ and N_T/N_C vs. N_R/N_C SD ($p=.10$) AND			
			$N_R/N_C - N_T/N_C > 0.15$	NOCN	$N_R/N_C - N_T/N_C > 0.30$	NOCN
Neanthes Growth	$M_C \leq 10\%$ and $MIG_C \geq 0.38$	$M_R \leq 20\%$ and $MIG_R \div MIG_C \geq 0.80$	$MIG_T \div MIG_C < 0.80$ and MIG_T vs. MIG_R SD ($p=.05$) AND			
			$MIG_T/MIG_R < 0.70$	NOCN	$MIG_T/MIG_R < 0.50$	$MIG_T/MIG_R < 0.70$

M = mortality
 N = normal larvae
 I = initial count
 MIG = mean individual growth rate (mg/individual/day)
 SD = statistically significant difference
 NOCN = no other conditions necessary

Subscripts:
 R = reference sediment
 C = negative control
 T = test sediment

9.4 REFERENCE SEDIMENT COLLECTION SITES

Bioassays must be run with a reference sediment which is well-matched to the test sediments for grain-size. **Table 9-2** contains information about each of the Puget Sound sites that are recommended for use. **Table 9-3** contains information about reference sites for Grays Harbor and Willapa Bay. Other reference areas may be utilized with DMMP review and approval if:

- biological tests are initially run using the proposed reference area along with an already recognized reference area.
- chemical (DMMP contaminants of concern) analysis is performed for the proposed area.

Table 9-2. Reference Sediment Collection Areas for Puget Sound.

	CARR INLET	SAMISH BAY	HOLMES HARBOR	SEQUIM BAY
Fines (%)	5-85	11-96	3-96	19-85
TOC (%)	0.2-11.8	0.4-29.0	0.2-31.0	2.3-2.7
Reference	PTI, 1991; SAIC, 2001	PTI, 1991; SAIC, 2001	PTI, 1991; SAIC, 2001	DAIS

Table 9-3. Reference Sediment Collection Sites for Grays Harbor and Willapa Bay

PARAMETER	STATION					
	3.9 MILE ODMDS	WBS5	WBS7	GHS4	GHS6	GHS7
Location	SE of 3.9 Mile Site ¹	Grassy Point	Bay Center	Stearns Bluff	Elk River	North Bay
GPS Latitude (WGS84)	46° 51.00'	46° 38.04'	46° 37.90'	46° 55.73'	46° 52.52'	47° 00.35'
GPS Longitude (WGS84)	124° 13.73'	124° 01.78'	123° 56.80'	123° 59.03'	124° 04.78'	124° 05.79'
Fines (%)	10	0	35-52	12	2	7-59
TOC (%)	0.10	0.02	0.51-1.0	0.25	0.06	0.15 - 1.1

Table adapted from *Grays Harbor and Willapa Bay Dredged Material Management Study: Expanded Reference Area Sediments* final report (SAIC, 1993)

¹ Station 4 from the 3.9-Mile ODMDS site.

The sampling protocol used for the collection of reference sediment can affect its performance during biological testing. The following guidelines should be followed when collecting reference sediments:

- Use experienced personnel.
- Follow PSEP protocols.
- Sample from biologically active zone.
- Avoid anoxic sediment below the Redox Potential Discontinuity (RPD) horizon.

- Use wet-sieving method in the field to target appropriate grain sizes.
- Fix sulfides sample with zinc acetate.

Wet-sieving in the field is imperative for finding a good grain-size match with the test sediment. Wet-sieving is accomplished using a 63-micron (#230) sieve and a graduated cylinder; 100 ml of sediment is placed in the sieve and washed thoroughly until the water runs clear. The volume of sand and gravel remaining in the sieve is then washed into the graduated cylinder and measured. This represents the coarse fraction; the fines content is determined by subtracting this number from 100. Because of the wide heterogeneity of grain size in the reference areas, it may be necessary to perform wet-sieving in several places before a reference sediment with the proper grain size is found. It is important that the sediment sample analyzed by wet-sieving is representative of the sediment that will be used for bioassays. Homogenization of the sediment prior to wet-sieving is recommended.

It should be noted that wet-sieving results will not perfectly match the dry-weight-normalized grain size results from the laboratory analysis, but should be relatively close (generally within 10%). It is requested that wet-sieving results be submitted along with the laboratory data so that a regression line for each embayment can be developed which more accurately predicts the dry-weight fines fraction from the wet-sieving results found in the field. Reference station coordinates should also be reported, with an accuracy of ± 3 meters.

In addition to wet-sieving in the field, reference sediments must be analyzed in the laboratory for total solids, total volatile solids, total organic carbon, grain size, ammonia and sulfides. The methods and QA guidelines used for analysis of sediment conventionals in test sediments should also be used for reference sediments.

9.5 FRESHWATER BIOASSAYS

In order to meet the requirements of the State of Washington's Sediment Management Standards as updated in 2013, freshwater bioassays used to assess toxicity of sediments in the DMMP program must include the following:

1. Two different test species: *Hyaella azteca* and *Chironomus dilutus*
2. A total of three endpoints
3. One chronic test: 20-day *Chironomus* or 28-Day *Hyaella*
4. One sublethal (growth) endpoint

Table 9-4 indicates which bioassay endpoints fall into which category. For freshwater bioassay test protocols, follow USEPA, 2000 and/or ASTM, 2010.

9.6 FRESHWATER BIOASSAY PERFORMANCE STANDARDS AND INTERPRETIVE CRITERIA

Freshwater biological tests are based on a comparison to control sediments, therefore it is not necessary to collect a reference sediment. This is primarily due to a lack of established reference sediment sites in freshwater areas of Washington State. Dredging projects wishing to use a reference sediment must have the location approved by the DMMP agencies prior to collection of the reference sediment. Use of reference sediment is strongly encouraged.

Table 9-4. Freshwater biological tests, species and applicable endpoints.

Species, biological test, and endpoint	Acute effects biological test	Chronic effects biological test	Lethal effects biological test	Sub-lethal effects biological test
Amphipod: <i>Hyalella azteca</i>				
10-Day mortality	X		X	
28-Day mortality		X	X	
28-Day growth		X		X
Midge: <i>Chironomus dilutus</i>				
10-Day mortality	X		X	
10-Day growth	X			X
20-Day mortality		X	X	
20-Day growth		X		X

9.6.3 Quality Control for Negative Control and Use as Reference Sediment

Negative control sediments are used in bioassays to check laboratory performance. Negative control sediments are clean sediment in which the test organism normally lives and which are expected to produce low mortality.

All freshwater bioassays have negative control performance standards that must be met (see **Table 9-5**). In the 10-day and 28-day *Hyalella* bioassay tests, mortality of the test organisms during the entire exposure period must be less than or equal to 20 percent. For the *Chironomus* 10-day test, mortality over the exposure period must be less than or equal to 30%, and less than or equal to 32% for the 20-day test. This represents a generally accepted level of mortality of test organisms under control conditions, indicating that the bioassay (in terms of test organism health) is considered a valid measure of effects of the test treatments. If control mortality is greater than the performance criteria, the bioassay test will generally have to be repeated, although that determination must be made in consultation with the agencies through the DMMO. Additionally, there are negative control performance criteria for the *Hyalella* 28-day and *Chironomus* 10-day and 20-day growth bioassays (see **Table 9-5**). Laboratories failing to achieve the control growth rate performance criteria may be required to retest. Since the negative control is used for test comparisons with freshwater bioassays, it is also advised to compare the grain size distribution of the control sediments to the test sediments.

9.6.4 Replication

For freshwater bioassays, eight replicates are run for each test sediment, as well as for the control sediment.

9.6.5 Positive Control

A positive control, or reference toxicant test, will be run for each bioassay. Positive controls are chemicals known to be toxic to the test organism. The positive control provides an indication of the sensitivity of the particular organisms used in a bioassay. Positive controls are performed

on freshwater spiked with the reference toxicant and compared with historical laboratory reference toxicity test results.

9.6.6 Water Quality Monitoring

Water quality monitoring of the overlying water should be conducted for freshwater bioassays. Daily measurement of temperature and dissolved oxygen should be conducted for the amphipod and midge tests. Conductivity, hardness and alkalinity should be measured at test initiation and termination for the amphipod and midge tests, if field conditions were measured during sampling. Monitoring of ammonia and total sulfides should be measured at test initiation and termination if either of these chemicals is suspected as being a problem (Ecology, 2008). Ammonia and sulfides values developed by Ecology as part of the Floating Percentile Model for freshwater sediment guidelines are used by the DMMP only to inform the need for bioassay purging. These values are:

- Ammonia: SL1/SQS=230; SL2/CSL = 300
- Total Sulfides: SL1/SQS= 39; SL2/CSL = 61

If ammonia and sulfides exceed these levels, the project proponent should coordinate purging protocols with the DMMP (see Section 9.8).

9.6.7 Freshwater Interpretive Criteria

The response of bioassay organisms exposed to composited sediment representing each DMMU will be statistically compared to the response of these organisms in the control sediment. **Table 9-5** specifies the bioassay performance criteria used for freshwater bioassays. These interpretive criteria were adopted at SMARM 2014 and revised at SMARM 2015 ([DMMP, 2015c](#)).

When any biological test exhibits a test sediment response that fails to meet the SL1/SCO criteria, the DMMU is judged to be unsuitable for unconfined open-water disposal in freshwater (see **Table 9-5**). Freshwater disposal sites are primarily dispersive. If non-dispersive disposal sites become available, the DMMP may evaluate sediments that fail SL1 criteria but pass SL2 criteria for in-water placement in a managed disposal site on a case-by-case basis.

The “one-hit/two-hit” interpretive criteria associated with marine sediments do not apply to freshwater sediments.

Table 9-5. Freshwater biological criteria (test performance standards) for each biological test.

Biological Test/ Endpoint ^a	Performance Standard ^b		Screening Level 1 (SL1)	Screening Level 2 (SL2)
	Control ^c	Reference		
<i>Hyalella azteca</i>				
10-day mortality	$M_C \leq 20\%$	$M_R \leq 25\%$	$M_T - M_C > 15\%$ and $M_T \text{ vs } M_C \text{ SD } (p \leq 0.05)$	$M_T - M_C > 25\%$ and $M_T \text{ vs } M_C \text{ SD } (p \leq 0.05)$
28-day mortality	$M_C \leq 20\%$	$M_R \leq 30\%$	$M_T - M_C > 10\%$ and $M_T \text{ vs } M_C \text{ SD } (p \leq 0.05)$	$M_T - M_C > 25\%$ and $M_T \text{ vs } M_C \text{ SD } (p \leq 0.05)$
28-day growth	$MIG_C \geq 0.15$ mg/ind	$MIG_R \geq 0.15$ mg/ind	$(MIG_C - MIG_T)/MIG_C > 0.25$ and $MIG_T \text{ vs } MIG_C \text{ SD } (p \leq 0.05)$	$(MIG_C - MIG_T)/MIG_C > 0.40$ and $MIG_T \text{ vs } MIG_C \text{ SD } (p \leq 0.05)$
<i>Chironomus dilutus</i>				
10-day mortality	$M_C \leq 30\%$	$M_R \leq 30\%$	$M_T - M_C > 20\%$ and $M_T \text{ vs } M_C \text{ SD } (p \leq 0.05)$	$M_T - M_C > 30\%$ And $M_T \text{ vs } M_C \text{ SD } (p \leq 0.05)$
10-day growth	$MIG_C \geq 0.48$ mg/ind	$MIG_R/MIG_C \geq 0.8$	$(MIG_C - MIG_T)/MIG_C > 0.20$ and $MIG_T \text{ vs } MIG_C \text{ SD } (p \leq 0.05)$	$(MIG_C - MIG_T)/MIG_C > 0.30$ and $MIG_T \text{ vs } MIG_C \text{ SD } (p \leq 0.05)$
20-day mortality	$M_C \leq 32\%$	$M_R \leq 35\%$	$M_T - M_C > 15\%$ and $M_T \text{ vs } M_C \text{ SD } (p \leq 0.05)$	$M_T - M_C > 25\%$ and $M_T \text{ vs } M_C \text{ SD } (p \leq 0.05)$
20-day growth	$MIG_C \geq 0.60$ mg/ind	$MIG_R/MIG_C \geq 0.8$	$(MIG_C - MIG_T)/MIG_C > 0.25$ and $MIG_T \text{ vs } MIG_C \text{ SD } (p \leq 0.05)$	$(MIG_C - MIG_T)/MIG_C > 0.40$ and $MIG_T \text{ vs } MIG_C \text{ SD } (p \leq 0.05)$

Notes:

M = Mortality; C = Control; R = Reference; T = Test; F = Final; MIG = Mean Individual Growth at time final; ind = individual; mg = milligrams; SD = statistically significant difference.

^a These tests and parameters were developed based on the most updated American Society for Testing and Materials protocols.

^b Reference performance standards are provided for sites where the department has approved a freshwater reference sediment site(s) and reference results will be substituted for control in comparing test sediments to criteria.

^c The control performance standard for the 20 day test (0.60 mg/individual) is more stringent than for the 10 day test and the agencies may consider, on a case-by-case basis, a 20 day control has met QA/QC requirements if the mean individual growth is at least 0.48 mg/individual.

9.7 ELUTRIATE BIOASSAY TESTING

The Tier 3 evaluation of dredged material in some cases may include bioassay testing of dredging elutriates to estimate water quality impacts ([USACE et al, 2009](#)). Elutriate testing for biological effects is not routinely required for regulated or federal dredging projects evaluated under CWA Section 404 for DMMP disposal. This test is conducted only when the Washington Department of Ecology requires it for assessment of potential water column toxicity effects relative to a particular chemical of concern.

*In the event that elutriate testing is required for marine sediments at the dredging site, the echinoderm/bivalve larval test will be conducted to evaluate water column effects. The appropriate assessment is described in the Sediment Evaluation Framework (SEF). More specificity on the serial dilution bioassay tests performed on the elutriate water can be found in the Inland Testing Manual (EPA/USACE, 1998, Sections 6.1 and 11.1). In the event that freshwater sediments at the dredging site require elutriate testing, and where salmonid species are present, elutriate testing should be conducted with rainbow trout (*Oncorhynchus mykiss*). The following species may be used for the larval water column bioassay test:*

- Echinoderm: *Dendraster excentricus* (marine)
- Bivalve: *Mytilus galloprovincialis* (marine)
- Rainbow trout: *Oncorhynchus mykiss* (freshwater)

9.8 AMMONIA AND SULFIDE NON-TREATMENT EFFECTS

The potential for ammonia and sulfides to complicate **marine** bioassay evaluations of dredged material has been addressed in the following DMMP clarification papers:

- [DMMP \(1993\)](#) - The *Neanthes* 20-day Bioassay – Requirements for Ammonia/Sulfides Monitoring and Initial Weight
- [DMMP \(2001b\)](#) - Reporting Ammonia LC50 data for Larval and Amphipod Bioassays
- [DMMP \(2002a\)](#) - Ammonia and Amphipod Toxicity Testing
- [DMMP \(2004a\)](#) - Ammonia and Sulfide Guidance Relative to *Neanthes* Growth Bioassay
- [DMMP \(2015b\)](#) – Modifications to Ammonia and Sulfide Triggers for Purging and Reference Toxicant Testing for Marine Bioassays

The 2015 clarification paper addresses data gaps and inconsistencies in the previous guidance and should be consulted for further details and background information on this topic. In summary, triggers for purging bioassay containers in order to reduce ammonia and/or sulfides prior to testing are given in Table 9-6. The trigger for purging was set to be equal to the No Observed Effects Concentration (NOEC) for the most toxic forms of ammonia and sulfides – unionized ammonia and hydrogen sulfide. Unionized ammonia and hydrogen sulfide concentrations must be derived from measurements of total ammonia and sulfides using test-specific pH, temperature, and salinity measurements. For ammonia, the trigger for conducting Reference Toxicant testing (Ref Tox) is set at half the lowest NOEC. Reference toxicant testing is not required for sulfides.

Table 9-6. Reference Toxicant and Purging Triggers for Marine Bioassays

Trigger	Bedded sediment tests				Larval tests	
	<i>Neanthes</i>	<i>Ampelisca</i>	<i>Eohaustorius</i>	<i>Rhepoxynius</i>	Bivalve	Echinoderm
Unionized Ammonia (mg/L) Ref Tox	0.23	0.118	0.4	0.2	0.02	0.007
Unionized Ammonia (mg/L) Purge	0.46	0.236	0.8	0.4	0.04	0.014
Hydrogen Sulfide (mg/L) Purge	3.4	0.0094	0.122	0.099	0.0025	0.01

9.8.1 Determining the Need for Purging or Ref Tox Testing:

The need for purging or Ref Tox testing should be conducted PRIOR to the commencement of actual bioassay testing. The following summarizes the recommended procedure (See [DMMP, 2015b](#) for details):

- 1. Measure bulk ammonia and sulfides in sediment.** Bulk ammonia and bulk sulfides measurements should be measured by the chemistry lab on composited sediment representing each DMMU. Exceptions to this procedure for total sulfides may be considered for sediment testing performed for both cleanup and DMMP characterization; and for projects where wood waste in new surface material may be an issue. In those cases, total sulfides should be performed on single cores.
- 2. Measure ammonia and sulfides in bioassay medium of exposure.** For those DMMUs that will undergo bioassays, ammonia and sulfides must be measured in the medium of exposure prior to running the bioassays. While bulk measurements made by the analytical laboratory can provide an early warning of potential non-treatment effects in bioassays, these measurements are not always predictive of the ammonia and sulfide concentrations to which bioassay organisms will actually be exposed. Aqueous concentrations measured by the bioassay lab are more meaningful in this regard.

For bedded sediment tests using *Neanthes*, *Eohaustorius* and *Rhepoxynius*, porewater is the medium of exposure. For the tube-building amphipod *Ampelisca*, as well as the bivalve and echinoderm species used in the larval development test, the overlying water is the medium of exposure.

Measurement of ammonia and sulfides in the medium of exposure can be accomplished by the bioassay lab by making measurements on two beakers for each DMMU, the first of which is set up in the manner that would be done for the amphipod bioassay and the second of which is set up as would be done for the larval bioassay. Since the juvenile infaunal bioassay is set up in the same way as the amphipod bioassay, the amphipod beaker is also predictive of ammonia/sulfides in the juvenile infaunal bioassay. In addition to ammonia and sulfides, pH is also measured. Unionized ammonia and hydrogen sulfide concentrations are calculated using the measured pH, plus the temperature and salinity that will be maintained during the bioassays.

3. **Prepare bioassays without purging.** If unionized ammonia and hydrogen sulfide concentrations are below the purging triggers in **Table 9-6**, or if any of the chemicals of concern exceeding SL are subject to significant loss or alteration of bioavailability during purging (to be determined in consultation with the DMMP agencies), set up the bioassays normally, without sacrificial beakers or purging. Run the ammonia reference toxicant test concurrently with a bioassay if the Ref Tox trigger is exceeded for the test organism being used.
4. **Prepare bioassays with purging.** If a purging trigger is exceeded for the species being used – and contaminant loss or alteration of bioavailability due to purging has been determined not to be a significant issue – prepare for purging. If the purging trigger for ammonia is exceeded, run the ammonia reference toxicant test concurrently.

9.8.2 Purging methods

For sediment toxicity testing, there are a variety of approaches used by regulatory agencies, project proponents and laboratories to purge samples. Purging is most often performed either by replacing overlying water twice a day plus continuous aeration, or by aeration alone. Once the unionized ammonia and/or hydrogen sulfide concentrations are below the trigger levels in **Table 9-6** for all test samples (labs should use the minimum purging required to bring concentrations below the trigger levels), the bioassay may be initiated. Each batch of test sediments must have associated and similarly purged control and reference sediments.

For further information on recommended purging methods and reporting of data see DMMP (2015b).

9.8.3 Case-by-case Determination to Allow Purging

The purging process may cause loss of more volatile/less hydrophobic COCs while less volatile compounds with a higher log K_{ow} remain associated with particles and dissolved organic matter. In addition, metals bioavailability and toxicity can be influenced by purging. Limited testing provided evidence that contaminant loss due to volatilization may not be an issue for the purging methods described above (DMMP, 2015b). The DMMP agencies will therefore continue to consider the specific contaminants triggering biological testing in decisions regarding purging. If contaminants may potentially be lost or their toxicity altered while purging for ammonia or sulfides, then purging may be disallowed or restricted in duration. Also, in some cases, ammonia or sulfides themselves may be contaminants of concern (e.g. new surface material containing wood waste) and purging may not be allowed. Purging is also not allowed for cleanup evaluations. For projects that include both cleanup and DMMP evaluation, side-by-side testing of both purged and non-purged sediments may be required.

9.8.4 Application of Purging Recommendations

The dredging proponent assumes the risk of dredged material being found unsuitable for open-water disposal if potential effects of ammonia and sulfides are not proactively addressed.

Proactively addressing ammonia and sulfides requires advanced planning. Sufficient volumes of sediment must be collected for sacrificial beakers; the pretesting and purging procedures must be included in the sampling and analysis plan; and holding times must be considered. The dredging proponent will need to balance the cost of these procedures against the cost of upland disposal of dredged material that fails toxicity testing due to non-treatment effects from ammonia/sulfides.

Ammonia and sulfides are more likely to be present in deeper sediments or sediments containing a significant fraction of organic material such as wood waste. Therefore, the type of sediment being tested will need to be assessed to determine the likelihood for elevated ammonia and sulfides. Initial bulk ammonia and sulfides testing by the analytical lab will also provide valuable information in this regard.

Alternative procedures from those described in this user manual may be proposed on a project-specific basis. Justification for the selected procedures must be clearly articulated in the sampling and analysis plan.

Close coordination with the DMMP agencies must be maintained throughout the process, from development of the pre-bioassay testing procedures in the sampling and analysis plan, to decision-making about purging and details of the purging procedure itself. All procedures must be approved by the agencies before the procedures may be performed.