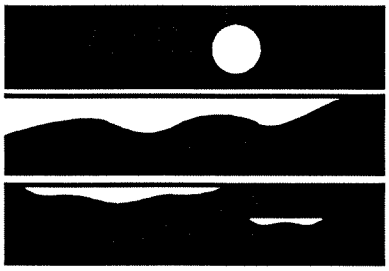

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Review of Sediment Management Standards Bioassay Protocols

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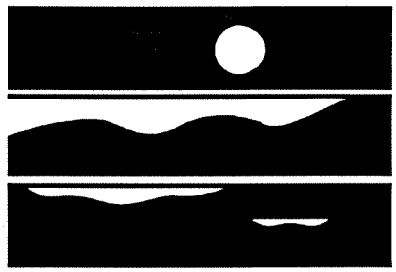

Review of Sediment Management Standards Bioassay Protocols

Produced for Ecology's Environmental Review / Sediment Section

by
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Olympia, Washington 98504-7710

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Table of Contents

List of Acronyms

ASTM	American Society for Testing and Materials
EC_{50}	Median Effective Concentration
EPA (U.S. EPA)	United States Environmental Protection Agency
LC_{50}	Median Lethal Concentration
NOAA	National Oceanic and Atmospheric Administration
PSDDA	Puget Sound Dredged Disposal Analysis
PSEP	Puget Sound Estuary Program
QA/QC	Quality assurance and quality control
SMS	Sediment Management Standards

Abstract

The Washington State Department of Ecology (Ecology) adopted the Sediment Management Standards (SMS) Chapter 173-204 WAC in March 1991, establishing Ecology's requirements for monitoring sediment quality in the state of Washington.

Biological effects criteria were established based on Amphipod, Larval, Juvenile Polychaete, and Microtox-Saline Extract sediment bioassays as identified in the Puget Sound Estuary Program (PSEP) Bioassay Protocols.

A review of the SMS bioassay suite was undertaken in preparation for a scheduled review and possible revision of the SMS in 1995.

Issues identified requiring attention included:

- Effects of non-treatment factors (grain size distribution, salinity, ammonia, and sulfides) on test outcome
- Need for review of the control performance standard and adoption of a reference sediment performance standard for Larval bioassays
- Need for review of the endpoints required for the Juvenile Polychaete test, and adoption of additional performance standards
- Problems with sensitivity of the Microtox-Saline Extract test

Recommendations for resolution of these issues are discussed.

Summary of Findings

A review of the existing suite of SMS bioassay test requirements, and the PSEP Protocols to meet those testing needs, has resulted in the following observations:

- The suite of SMS bioassays generally offers an appropriate variety of organisms and sensitivity to sediment toxicity
- The Microtox Saline Extract test may be limited in its utility for SMS testing
- The PSEP Protocols are technically sound, and recommendations for refinements in their application for SMS testing are minor, primarily requiring water quality monitoring which is optional in the Protocols
- Some SMS bioassay endpoints and performance standards require updating to reflect observations made in Puget Sound bioassays and technical advances in interpretation of test results
- Addition of two species to the Amphipod Bioassay is expected to resolve problems with *Rhepoxynius abronius'* sensitivity to test sediment grain size and salinity

Recommendations

- The SMS should include *Ampelisca abdita* and *Eohaustorius estuarius* in the Amphipod Bioassay as alternate species to be used when sediment salinity and/or grain size are outside the range of tolerance for *Rhepoxynius abronius*. *R. abronius* should remain the required species for Amphipod testing. Substitutions, if appropriate, should be approved by Ecology prior to testing. Use of the two alternative species should follow the guidance provided in the draft PSEP Bioassay Protocols. *A. abdita* should be used if sediment fines equal or exceed 60%. *E. estuarius* is recommended for situations in which salinity is less than 25 ppt and sediment fines are less than 60%.

- The SMS should require water quality monitoring for sulfides and ammonia in test systems for Amphipod, Larval, and Juvenile Polychaete bioassays, as recommended by the PSEP protocols. Additional warning limits for sulfides and ammonia should be developed as supporting data become available.

- The SMS should adopt a more stringent control performance standard for Larval Bioassays, requiring at least 70% normal survival for validation of tests.

- The SMS should include *Strongylocentrotus drobachiensis* as one of the species recommended for Larval bioassays.

- The SMS should be modified to replace biomass with growth rate as the required sublethal endpoint for the Juvenile Polychaete test. In addition, the SMS should include mortality as a clearly defined endpoint for designation of sediments.

- A growth rate control performance standard of 0.65 mg/day has been recommended for the Juvenile Polychaete test. The SMS should evaluate available Juvenile Polychaete growth data to determine if this limit is consistent with results observed in Puget Sound. Reference sediment performance standards should be developed for growth rate and for mortality. The established control performance standard of $\leq 10\%$ mortality should be maintained.

- Microtox Saline Extract testing should be reviewed for its relative usefulness in measuring marine sediment toxicity. Ongoing investigations into Microtox methods should be monitored to determine if another technique might be more useful for SMS sediment testing.

Background

The Washington State Department of Ecology (Ecology) adopted a state rule in March 1991 addressing sediment quality: the Sediment Management Standards (SMS), Chapter 173-204 WAC. The SMS were reviewed and approved by the U.S. Environmental Protection Agency (EPA) Region 10 pursuant to Section 303 of the Clean Water Act. This allows Ecology to monitor sediment quality in the state of Washington, requiring adherence to specific criteria for discharge source control and cleanup of contaminated sediments.

The Environmental Review/Sediment Section is responsible for implementation and enforcement of the SMS, and it plans to revise the SMS in 1995. This provides an opportunity for review of the bioassay suite and protocols currently used, to determine the need for revision. The Environmental Review/Sediment Section has requested assistance from the Environmental Investigations and Laboratory Services Program in conducting this review of the SMS bioassay suite.

The Work Plan for Review of Sediment Management Standards Bioassay Protocols outlines the following:

Task 1. Review existing SMS bioassay protocols and recommend changes if needed

Task 2. Provide recommendations for data deliverables to be included in sediment bioassay reports submitted to Ecology

Task 3. Identify and evaluate bioassays not presently included which should be considered for use in the SMS

This report describes the findings of Task 1. The primary objective of Task 1 is to review the protocols currently used for the existing suite of SMS bioassays -- with attention to application, performance and interpretation of results from each test -- and to make recommendations for modifications if warranted.

Existing Suite of SMS Bioassays

The SMS rule provides for the use of biological tests to confirm chemical criteria designation of marine sediments as having adverse effects on biological resources. These biological effects criteria also apply to defining sediment zone impacts and sediment cleanup criteria. Marine tests included in the SMS are: the ten-day Amphipod Sediment Bioassay; any one of the Echinoderm Embryo or Bivalve Larvae Bioassays; the twenty-day Juvenile Polychaete Bioassay; and the Microtox Bioassay - Saline Extract. Currently the use of Puget Sound Estuary Program (PSEP) protocols is required when conducting Puget Sound Marine Sediment bioassays under the SMS (Ecology, 1991). These biological tests and their endpoints are summarized in Table 1.

Concurrent with the review being conducted for Task 1 of this project, PSEP was proceeding with an evaluation and revision of the Bioassay Protocols. The Technical Review Committee -- including representatives from Ecology, EPA, the Army Corps of Engineers, NOAA, and environmental consultants -- has produced a draft document which will be submitted to the PSEP Management Committee for approval early in 1995 (PSEP, 1994). The PSEP evaluation and revision has produced technically sound laboratory test methods, which are reviewed here, as they apply to the SMS.

Below is a discussion of the individual SMS bioassays which covers application, problems encountered, and recommended solutions. Many of the recommendations are based upon the most recent PSEP draft bioassay protocols (PSEP, 1994). A list of contacts, individuals whose expertise is in these bioassays and their regulatory application, is in Appendix I.

Table 1. Summary of Bioassays included in the Sediment Management Standards.

Bioassay	Species	Exposure	PSEP Endpoints	SMS Required Endpoints	Significant Responses[a]	Other Factors
Amphipod	*Rhepoxynius abronius* *Ampelisca abdita*[b] *Eohaustorius estuarius*[b]	10 days Static	Mortality, emergence, reburial	Mortality	181 of 674 samples	Sensitivity to grain size and salinity
Larval	*Crassostrea gigas* *Mytilus edulis*	48 hours Static	Mortality, abnormality, effective mortality (combined mortality/abnormality)	Normal survival (combined mortality/ abnormality)	17 of 56 samples[c]	Sensitivity to ammonia, sulfides, and grain size; Seasonal variability in test response
	Strongylocentrotus purpuratus *Dendraster excentricus*	48-96 hours Static	Mortality, abnormality, effective mortality (combined mortality/abnormality)	Normal survival (combined mortality/ abnormality)	79 of 205 samples[c]	
Juvenile Polychaete	*Neanthes arenacoedentata*	20 days Static renewal with feeding	Mortality, total and individual biomass, growth rate	Mean (individual) biomass	12 of 71 samples	Sensitivity to ammonia and sulfides
Microtox - Saline Extract	*Photobacterium phosphoreum*	15 minutes	Decrease in luminescence, EC_{50}	Luminescence	29 of 50 samples[d]	Enhanced bioluminescence; Lack of toxic response

[a] Statistically significant adverse test responses from SEDQUAL Database (Gries, pers. comm., 1994); Juvenile Polychaete data are from PSDDA DAIS Database (Littleton, pers. comm., 1994)

[b] Added in 1994 review of PSEP Protocols

[c] Data based on Abnormality Endpoint

[d] Most data are from 1986-1988; much of the data since 1988 show enhanced bioluminescence, making interpretation difficult

Marine Amphipod Bioassay

Application

The Amphipod test specified in the SMS is the ten-day mortality sediment bioassay, using *Rhepoxynius abronius*. The animals are exposed in a static test system composed of an aliquot of sediment overlain with clean dilution water. The PSEP Protocol test endpoints are mortality, daily emergence, and ability of the organisms to rebury after ten days exposure to sediments.

The SMS required endpoint is mortality. Under the existing SMS rule, a sediment is determined to have adverse effects on biological resources when mean amphipod mortality exceeds 25% ($p \leq 0.05$) and is statistically greater than that of the reference sediment. The Amphipod test is considered an acute effects test under the SMS.

R. abronius is one of the first organisms to be used for marine sediment testing on the Pacific Coast, first introduced by Swartz *et al.* (Swartz, 1979). It has become a standard in marine sediment testing, and a number of other freshwater and marine tests have been modeled after this procedure. The Marine Amphipod test has been used in Puget Sound to evaluate toxicity in urban embayments, at Superfund sites, for ambient monitoring, and for regulatory purposes. It is currently used by virtually all agencies involved in Puget Sound sediment testing. In general, the Marine Amphipod test has been found to be reproducible and ecologically relevant and has consistently performed well over the years of its use. Based on Ecology's SEDQUAL data, approximately one quarter of the Puget Sound sediments tested using this organism have shown significant toxicity (Table 1.)

Amphipod Issues

A review of the PSEP Protocols and SMS identified the following issues:

1. Non-Treatment factors which may result in Type I error (false positive effects)
   - Salinity Tolerance
   - Grain Size Distribution
   - Presence of Ammonia and/or Sulfides

2. "Amphipod" is the only organism in the bioassay suite that is included in the SMS Definitions (WAC 173-204-200).

Non-Treatment Factors

Salinity Tolerance and Grain Size Distribution

R. abronius is sensitive to salinities less than 25 ppt (ASTM, 1992). The PSEP protocols allow for adjustment of low-salinity test sediments, but caution against the practice because adjustment may impact toxicological properties of the sediment.

R. abronius has been reported to be sensitive to grain sizes of fine and very coarse material (DeWitt *et al.*, 1988; DeWitt, pers. comm., 1995). A Puget Sound Dredged Disposal Analysis (PSDDA) review of dredged material evaluations has confirmed that high percentages of fine grained sediments (greater than about 60 percent clay/silt) can lead to false positive effects when tested against *R. abronius* in Puget Sound (Kendall, 1993).

Ammonia and Sulfides

Ammonia and sulfides are known to be toxic to aquatic organisms; however, their role in sediment toxicity testing is poorly defined and has only recently become a focus of investigation. Interpretation of bioassay results is confounded by a lack of information on ammonia and sulfide toxicity, and conditions under which their presence may impact the outcome of testing.

Results of sediment testing in Puget Sound suggest that ammonia is only occasionally a confounding factor in testing of dredged materials, less often in testing of surficial sediments (Kendall, pers. comm., 1994). Investigators locally and nationally are evaluating concentrations of these compounds and conditions under which toxicity is likely to be affected. An upcoming report on investigations for the Port of Seattle is expected to offer insight into mechanisms of ammonia and sulfide toxicity in marine sediments in general, and Puget Sound in particular.

Debate continues about how to measure these compounds in test systems to best correlate ammonia and sulfide concentrations with test organism response. Concentrations in overlying and in interstitial water may each correlate with toxicity, but the significance of each is likely to be species specific (Long, 1994). For example, burrowing organisms, such as *R. abronius*, may be more impacted by interstitial concentrations; tube dwellers, such as *A. abdita*, may be more affected by overlying water.

Amphipod Definition

The Definitions section of the SMS includes a definition for "Amphipod" but not for the remainder of the organisms in the bioassay suite. It would be more consistent to either

exclude "Amphipod" or include definitions for all test organisms. If it is decided to include test organisms in the Definitions section, the required test species should be specifically cited. The present definition says, "e.g., *Rhepoxynius*," giving the mistaken impression that a number of Amphipod species may be used for testing. The SMS is actually quite specific in its Amphipod species requirement.

PSEP Recommendations

Non-Treatment Factors

Salinity and Grain Size

The Technical Review Committee identified non-treatment factors as important considerations in Amphipod testing. To address salinity and grain size issues, the draft PSEP Protocol for the Marine Amphipod Bioassay was expanded to include two additional species, *Ampelisca abdita* and *Eohaustorius estuarius*. *A. abdita* is found at salinities ranging from 10 to 35 ppt, and has survived well (>94%) in testing with grain size from 90% silt-clay to 86% coarse-medium sand. *E. estuarius* is collected at sites where the annual range of salinities is near 0 to 35 ppt. Testing has shown the organism to be tolerant of a wide range of grain size (>90% survival in 80% silt-clay, and in sandy sediments as well). (ASTM, 1992). Both species have well-established protocols and have been extensively used for testing around the U.S. *E. estuarius* is native to Washington State waters; *A. abdita* is normally considered an East Coast species, although it has been collected in San Francisco Bay. The PSEP protocols define conditions under which these species may be substituted in the Marine Amphipod test, when grain size and/or salinity of test sediments exceed *R. abronius*' tolerance.

The three Amphipod species are similar in their responses to whole sediment toxicity. While *R. abronius* is generally the most sensitive, *E. estuarius* is considered only slightly less sensitive than *R. abronius* to contaminants (ASTM, 1992). In a review of published results for the three species tested against cadmium and fluoranthene reference toxicants, PSDDA concluded all were appropriately sensitive for Puget Sound sediment testing and were relatively similar in their responses to whole sediment toxicity (Kendall, 1993).

Ammonia and Sulfides

Reviewers determined that available data are not sufficient to establish warning limits for ammonia and sulfides for the PSEP Amphipod bioassay. Presently the Protocols suggest measurement of these two compounds in the overlying water of the test system, but these measurements are optional. Most laboratories, however, submit ammonia and sulfide

results as a matter of course, recognizing their potential importance in interpretation of test results.

Recommendations for SMS

Non-Treatment Factors

Salinity and Grain Size

Ecology has identified the need for alternative species to *R. abronius* in certain instances under the SMS, and has made provision for approval of substitutions on a case-by-case basis (Betts, 1993). The rule provides for use of alternate technology when it is determined to improve monitoring capability. However, the process is relatively rigorous and would be facilitated by specifying the use of *A. abdita* or *E. estuarius* in the SMS as options, when grain size and/or salinity of test sediments exceed the tolerances of *R. abronius.*

Use of the two alternative species should follow the guidance provided in the draft PSEP Bioassay Protocols. *R. abronius* should continue to be the required Amphipod species. If substitutions are appropriate, they should be approved by Ecology prior to testing. *A. abdita* is an appropriate substitute for *R. abronius* if sediment fines equal or exceed 60%. *E. estuarius* is recommended only for low salinity situations, in which salinity is less than 25 ppt and sediment fines are less than 60%.

Ammonia and Sulfides

A review of recent research results will be necessary to determine if data on ammonia and sulfide toxicity are sufficient to establish warning limits in the PSEP bioassay protocols. Limits will not be available for inclusion in the present review of the Protocols. Recognizing the importance of ammonia and sulfide to test interpretation, and with a view toward development of warning limits, however, ammonia and sulfide analyses should be a requirement for amphipod bioassays under the SMS.

Amphipod Definition

"Amphipod" should either be removed from the definition section of the SMS, or entries should be included for the remainder of the test organisms to enhance consistency of the document. If the latter option is selected, the specific Amphipod species allowed should be listed in the definition (i.e., *R. abronius, A. abdita, E. estuarius*).

Larval Bioassays

Application

The Larval Bioassays specified by the SMS are those identified in the PSEP Protocols as the Bivalve Larvae Bioassay and the Echinoderm Embryo Bioassay. The two bivalve species used are the pacific oyster, *Crassostrea gigas*, and the bay mussel, *Mytilus edulis*. The Echinoderms are the purple sea urchin, *Strongylocentrotus purpuratus*, and the sand dollar, *Dendraster excentricus*. Newly fertilized eggs are exposed in a test system composed of an aliquot of test sediment, shaken with clean dilution water. At test termination (approximately 48 hours for the bivalves and 48-96 hours for the Echinoderms) surviving embryos are examined for normal development. PSEP endpoints are mortality, abnormality, and effective mortality (combined mortality and abnormality).

The SMS required endpoint is normal survival, a combined mortality/abnormality endpoint that compares the percent of survivors showing normal development at the time of termination to the number inoculated at test initiation. Under the existing SMS rule, a sediment is determined to have adverse effects on biological resources when mean normal survivorship is less than 85% of that of the reference sediment, and the difference is statistically significant ($p \leq 0.05$). The Larval test is considered an acute effects test under the SMS.

The bivalve larvae bioassay has been used in Washington State since the early 1970s and was standardized nationally by ASTM in 1980 (ASTM, 1989). The Echinoderm test was described by Dinnel and Stober (1985), and has been standardized by ASTM, as well (ASTM, 1994). Both tests have been modified for use in sediments. The Protocols for Bivalve and Echinoderm Sediment testing have been in use in Puget Sound since 1986. The ASTM Sediment Subcommittee (E47) is presently developing standard guidance annexes for these organisms.

The four test species can all be collected in Washington State; however, since testing is dependent on successful spawning and fertilization, it is often necessary to import animals for out-of-season testing. Such importation is the subject of strict regulation by the Department of Wildlife to limit the possibility of release of disease or parasites into native stocks.

The Larval tests are used locally and nationally for regulatory purposes, for dredged material disposal regulation, and for ambient monitoring of sediments. These bioassays have been found to be sensitive and ecologically relevant in Puget Sound testing. A

review of Ecology's SEDQUAL data indicates about one third of Larval bioassays have shown significant toxicity to test sediments (Table 1).

Larval Issues

A review of the PSEP Protocols and the SMS identified the following issues:

1. Non-Treatment Factors which may result in Type I error (false positive effects)
2. Need for update of Control Performance Standard
3. Need for Reference Performance Standard
4. Inclusion of *Strongylocentrotus drobachiensis* as a test organism option

Non-Treatment Factors

The recent focus of attention on non-treatment factors, and their impact on the outcome of sediment bioassays, has extended to the Larval tests, as well. The U.S. Army Corps of Engineers evaluated PSDDA bioassay results and found concentrations of ammonia to be statistically correlated to Echinoderm mortality, when chemicals of concern were not elevated in Puget Sound test sediments. Subsequent testing has shown that bivalves and Echinoderms exhibit lethal and sublethal responses to varying concentrations of ammonia, and has defined threshold levels at which false positives due to ammonia toxicity may occur in sediment testing. Aeration was found to mitigate the toxic effect of ammonia in test chambers (U.S. EPA, 1993).

Grain size distribution of test sediments have also been shown to impact bioassay results. Oyster larvae may be sensitive to predominately fine-grained sediments (>50% fines); however, sand dollars do not appear to be (U.S. EPA, 1993).

Update of Control Performance Standards

The SMS defines a control performance standard of at least 50% normal survival for larval tests. At the time the SMS was adopted, the PSEP performance standard was considerably more stringent, allowing not more than 30% mortality and 10% abnormality in controls. The difference in the two standards results from the fact that at the time the SMS standard was established, high control mortalities were consistently being observed in bivalve testing. PSDDA and SMS limits were defined to reflect this phenomenon, and to add flexibility to ensure successful Larval tests.

A PSDDA review of five years of test data, however, has shown that most of the Larval tests in their data base would have met a control performance standard of 70% normal

survival (Fox and Littleton, 1994). Based on this information, it would be appropriate to adjust the SMS limit to the more stringent standard.

Lack of Reference Sediment Performance Standard

The SMS does not define a Larval bioassay performance standard for reference sediments. As a result there is inherent uncertainty in using results of reference sediment bioassays to test for significance of biological effects. The historical variability exhibited, especially in the Bivalve mortality endpoint, has made development of a performance standard difficult, using either mortality or a combined endpoint (PSEP, 1991b).

The PSDDA agencies initially established a guideline for reference sediment performance at 20% effective mortality, that is, the percent of abnormal survivors (normalized to control response). That guideline has been revised by PSDDA because of the large number of tests which have been invalidated. The interim guideline adjusts the reference sediment performance standard to 35% effective mortality (normalized to control response), and includes standard deviation performance standards for reference and test sediments, to assure a power of at least 0.60 for significance testing. The PSDDA guideline appears promising; however, it is still undergoing technical review at this time.

Inclusion of *Strongylocentrotus drobachiensis*

The SMS presently limits Larval testing to four bivalve and Echinoid species. The PSEP Protocols include an additional Echinoderm, the green sea urchin (*Strongylocentrotus drobachiensis*). *S. drobachiensis* has been shown to be similar in sensitivity to the other Echinoid species in toxicity testing (Dinnel and Stober, 1987; Environment Canada, 1992).

Success in Larval testing is often seasonal, depending on the spawning condition of organisms and the quality of gametes for fertilization. Outside of an optimal period for each organism, spawning may be difficult, and quality of gametes and fertilization poor.

The green sea urchin normally spawns during the winter in Puget Sound, at a time when the SMS species are unlikely to be suitable for testing. Addition of this species to the SMS suite of Larval test organisms would improve the likelihood of successful Larval tests year round, and limit the need for importing out-of-state organisms for out-of-season testing.

PSEP Recommendations

Non-Treatment Factors

The PSEP Protocols now include interim warning limits for ammonia in bioassay test systems, and require aeration when ammonia and/or sulfides are present. Test data should be qualified as possible false positives if the oyster-specific threshold value of 0.13 mg/L unionized ammonia is exceeded. For the echinoderm bioassay, the ammonia testing criterion is 0.014 mg/L unionized ammonia; data are qualified as possible false positives if unionized ammonia values are equal to or greater than 0.04 mg/L. However, monitoring of ammonia and sulfide concentrations is optional under the PSEP Protocols.

To address grain size issues, PSEP cautions against use of *C. gigas* in testing sediments known to have a high proportion of clays and silts, and suggests use of *D. excentricus* under those conditions.

Update of Control Performance Standard

The PSEP control performance standard for Bivalve and Echinoderm testing has been adjusted from a minimum of 70% survival and no more than 10% abnormality, to 70% normal survival (that is, the percent of organisms inoculated into the test system that are normally developed at termination of testing).

Inclusion of *Strongylocentrotus drobachiensis*

The PSEP Protocols include *S. drobachiensis* as one of the three species recommended for Echinoderm sediment testing in Puget Sound.

Recommendations for SMS

Non-Treatment Factors

The PSEP Protocols have established interim warning limits for ammonia, and require aeration of test solutions if ammonia or sulfides are measured or suspected. However, water quality monitoring for ammonia and sulfide is optional. Recognizing the potential impact of these two compounds on Larval Bioassay test results, monitoring for ammonia and sulfides as recommended under the PSEP Protocols should be required for SMS Larval bioassays.

Update of Control Performance Standard

Available data support the adoption of a control performance standard of 70% normal survival for Larval bioassays under the SMS. The more stringent control performance standard would also make SMS testing consistent with that of PSEP, as well as PSDDA and ASTM.

Lack of Reference Sediment Performance Standard

Development of a reference sediment performance standard for Larval bioassays would be an important addition to the SMS. While a definitive standard is elusive at this time, PSDDA has developed an interim guideline that shows promise. The interim guideline adjusts the reference sediment performance standard to 35% effective mortality (normalized to control response), and includes standard deviation performance standards for reference and test sediments, to assure a power of at least 0.60 for significance testing. This would increase the number of valid reference sediments results, and therefore require fewer retests. The guidelines require further investigation from the technical workgroup, including a statistician, before permanent standards are set. Ecology should test this guideline against historical Larval test results to determine whether it would meet the SMS testing needs.

Inclusion of *Strongylocentrotus drobachiensis*

The SMS should include *S. drobachiensis* as one of the species recommended for Larval bioassays. The species exhibits similar sensitivity to the two Echinoderms presently recommended. Addition of *S. drobachiensis* to the suite would increase the options for successful testing with locally collected organisms, by adding to the collective optimal spawning season.

Juvenile Polychaete Bioassay

Application

The Juvenile Polychaete test is conducted using laboratory cultured juveniles of the marine Polychaete *Neanthes arenaceodentata.* The animals are exposed in a static test system similar to that used for the Amphipod test. During the 20-day exposure, the animals are fed to ensure adequate nutrition for survival and growth. The PSEP Protocol endpoints are mortality, total and individual biomass, and growth rate.

The SMS required endpoint is mean (individual) biomass. Under the existing SMS rule, a sediment is determined to have adverse effects on biological resources when mean biomass is less than 70% of that of the reference sediment, and the difference is statistically significant ($p \leq 0.05$). The Juvenile Polychaete test is considered a chronic effects test under the SMS.

The species originates from a California embayment. It has not been collected in Puget Sound. To obtain organisms for testing, they must be cultured in-house or imported from a single culturist in California. *N. arenaceodentata* is considered an exotic species, and importation for testing is restricted under Department of Wildlife regulations which were previously noted under "Larval Bioassays."

N. arenaceodentata is well-established as a sediment test organism. The species has been used for acute, chronic, and life-cycle sediment testing since the mid-sixties (ASTM, 1994a). Juvenile Polychaete tests are used nationally for regulating dredged material disposal in coastal areas by the Army Corps of Engineers. Early regulatory tests were based on an acute (10-day mortality) endpoint. A protocol for the 20-day biomass (growth) test was developed for Puget Sound use in 1991, sponsored in part by Ecology's Sediment Management Unit (PTI, 1989; PTI, 1991). A guidance document for marine sediment tests using juvenile Polychaetes was recently adopted by ASTM (ASTM, 1994b).

While some agencies around the U.S. still use the acute test, virtually all Puget Sound Juvenile Polychaete testing is now based on the 20-day growth test. The 20-day growth test has filled a void in marine sediment testing, where few standardized chronic bioassays are available. In Puget Sound, the Juvenile Polychaete biomass test is used for regulation of sediments for dredged material disposal, and in studies monitoring sediment toxicity. PSDDA initiated use of the test beginning in 1992, for a two-year trial period. PSDDA's DAIS database lists 12 of 71 sediments showing statistically significant adverse effects in Juvenile Polychaete testing (Table 1).

Juvenile Polychaete Issues

A review of the PSEP Protocols and the SMS has identified the following issues:

1. Non-Treatment Factors which may result in Type I error (false positive effects)
   - Ammonia and Sulfides

2. Need for update of Measured Endpoint
   - Growth Rate
   - Mortality

3. Need for update of Performance Standards
   - Growth Rate
   - Mortality

Non-Treatment Factors

Ammonia and Sulfides

Ammonia and sulfide have been shown to affect results of Juvenile Polychaete tests, particularly related to the sublethal endpoint. There are not yet enough data for models to predict toxicity at a given concentration; however, research at the Waterways Experiment Station (WES) has allowed Dillon *et al.*, 1993, to develop warning limits for both compounds.

Update of Measured Endpoints

The SMS uses two different endpoints for the Juvenile Polychaete test, depending on the application. The required endpoint for reference and test sediments, and the basis for significance testing, is mean individual biomass. The control performance standard is based on mortality. Biomass is generally considered to be the more sensitive of the two endpoints; however, at the inception of the SMS the database was inadequate for use as the foundation for a biomass-based control performance standard.

Growth Rate

Recent work at WES has resulted in a refinement of the 20-day Juvenile Polychaete test, the addition of the growth rate endpoint (Dillon *et al.*, 1993). Variability in test results often arises from differences in sizes of worms at initiation of individual tests. Calculation of the growth rate (expressed as mg of dry weight increase per day) functions to normalize biomass to initial worm size, making intra- and interlaboratory results comparable. Limiting variability serves to increase the utility of the data, improving confidence in

evaluation of test and reference sediments, and providing a solid basis for developing test performance standards. Further studies at WES have confirmed the earlier work, and provide interpretive guidance for use of the growth endpoint for Juvenile Polychaete testing (Moore and Dillon, 1993).

Mortality

Mortality is not represented in any SMS endpoints for reference or sample sediments for Juvenile Polychaete bioassays. The present required endpoint (individual biomass) and the proposed endpoint (growth rate) are both based on individual (mean) weights; neither of these endpoints integrate survival into its calculation. Not only is a valuable regulatory tool being under-utilized, but disregarding mortality as a test endpoint increases the potential for Type 2 error (false negative effects). Significant mortality in tests should be considered evidence of toxicity, in order to avoid situations in which robust worms survive in test sediment in which significant mortality occurs.

The Juvenile Polychaete test was selected for the SMS suite as an indicator of chronic toxicity, with mortality considered a secondary issue, evaluated based on best professional judgement of the reviewers. The mortality endpoint would be a more functional regulatory tool if it were clearly defined for designation of sediments.

A combined endpoint, as is used for Larval testing, may be possible but is unrealistic at this time due to resource requirements for development. A feasible alternative is to evaluate each endpoint separately, setting control and reference sediment performance standards for each. Hypothesis testing would then be used to determine the statistical significance of each endpoint in samples, relative to the reference sediment. Statistically significant mortality in a sample makes it unnecessary to test for growth differences. If mortality is significant, the more sensitive sublethal effects in a population can be reasonably assumed.

Update of Performance Standards

The Juvenile Polychaete performance standards and biological effects criteria are all based on either mortality or biomass. The control performance standard was developed at a time when there were not yet enough data to establish a biomass-based standard. Instead mortality is the basis for the control standard. This produces a potential gap in quality control since biological effects criteria and reference sediment performance standards use biomass endpoints. Controlling only for mortality could mask problems in the test system affecting the (biomass) outcome of the test. Conversely, controlling only for biomass in performance standards and test sediments leaves mortality unaccounted for.

Growth Rate

Adoption of the growth rate endpoint mandates development of new growth rate based performance standards for Juvenile Polychaete testing. Based on WES's development of the growth rate endpoint, Moore and Dillon, 1993, have shown that growth rates ≥0.65 mg/day resulted in adequate survival and reproduction of *N. arenaceodentata*. This limit could be the basis for the SMS control performance standard if it proves to be consistent with data produced in Puget Sound testing. Growth rates here may be different since parts of the PSEP test method, such as feeding requirements, are substantially different from those used by WES. An evaluation of existing Juvenile Polychaete data would be necessary to see if the WES limit is consistent with that observed in Puget Sound. A similar data review would be required for development of a reference sediment performance standard for growth rate.

Mortality

Adoption of a mortality endpoint for Juvenile Polychaete testing would mandate development of mortality-based performance standards. The existing control performance standard of ≤10% mortality is appropriate. Ideally, the reference sediment performance standard would be consistent with those developed for reference sediment mortality in the remainder of the SMS bioassay suite. However, a review of existing Juvenile Polychaete data would be required to substantiate the standard.

PSEP Recommendations

Non-Treatment Factors

Ammonia and Sulfides

The PSEP Protocols now include water quality monitoring limits for unionized ammonia (>0.7 mg/L), and total sulfides (>5 mg/L). Increased water quality monitoring is suggested when concentrations exceed the specified limits at test initiation. Monitoring of ammonia and sulfide concentrations are not required, only recommended by the PSEP Protocols.

Update of Measured Endpoint

The review of the PSEP Protocols introduced the growth rate endpoint to the Juvenile Polychaete test.

Update of Performance Standards

Performance Standards are not addressed in PSEP for any of the Juvenile Polychaete endpoints.

Recommendations for SMS

Non-Treatment Factors

Ammonia and Sulfides

The addition of warning limits for ammonia and sulfides to the PSEP protocol provides much needed guidance for conduct of the Juvenile Polychaete bioassay and for interpretation of test results. The PSEP Protocols now include water quality monitoring limits for unionized ammonia (>0.7 mg/L) and total sulfides (>5 mg/L). The presence of ammonia and sulfide is important to the outcome and interpretation of bioassay results. Therefore, water quality monitoring for ammonia and sulfide, as recommended in the PSEP protocols, should be required for SMS Juvenile Polychaete bioassays.

Update of Measured Endpoint

The biomass endpoint should be replaced by growth rate as the SMS required sublethal endpoint for the Juvenile Polychaete test. Additionally, the SMS should include mortality as a clearly defined endpoint for designation of sediments. Adverse effects would be determined by hypothesis testing of sample relative to reference mortality for each endpoint. Statistically significant mortality is sufficient to designate a test sediment, since the consequences of lethality immediately prevail over any sublethal effects.

Update of Control Performance Standard

A growth rate control performance standard of 0.65 mg/day has been recommended for the Juvenile Polychaete test. An evaluation of existing Juvenile Polychaete data would be necessary to ensure this limit is consistent with results observed in Puget Sound. A reference sediment performance standard would be derived from a similar data review.

The existing control performance standard of ≤10% mortality should be maintained. A reference sediment performance standard should be developed from existing data, to serve as a yardstick for evaluating mortality in test sediments.

Microtox Bioassay - Saline Extract

Application

The Microtox Bioassay tests the bioluminescent response of a marine bacterium, *Photobacterium phosphoreum*, to a saline extract of the sediment. Luminescence is measured before and after a 15-minute exposure to the extract. Decrease in light output is assumed to indicate toxicity of the sediment. For the PSEP Protocol, the percent decrease in luminescence and the EC_{50} for the test dilution series is reported.

The SMS endpoint is decreased luminescence after a 15-minute exposure. While PSEP is followed for the analysis, in this instance the SMS requires an alternative endpoint that is not specified by the Protocols. Rather than using PSEP's EC_{50} or percent decrease in luminescence, the SMS calls for mean light output, thereby simplifying the statistical comparison. Under the existing SMS rule, a sediment is determined to adversely affect biological resources when the mean light output of the highest concentration of test sediment is less than 80% of that of the reference sediment, and the two means are statistically different ($p \leq 0.05$).

The Microtox test is considered a surrogate chronic effects bioassay under the SMS. This designation was adopted and based on four considerations:

- Few standardized marine sediment chronic test methods were available
- A review of Apparent Effects Threshold (AET) data for Microtox identified the Microtox Saline Extract test to be among the most sensitive of known biological indicators, acute or chronic
- A precedent established by the PSDDA Agencies recognized Microtox Saline Extract as a chronic surrogate
- The public review process validated the need for additional chronic sublethal bioassays for sediment source control and cleanup

Microtox Saline Extract procedures have been used to test marine sediments in Puget Sound since 1986. Puget Sound regulatory agencies -- notably EPA, Ecology, and the Army Corps of Engineers -- included Microtox in their suites of bioassay tests. Early sediment tests showed the method to be very sensitive to toxicity relative to other tests in the suite.

Since 1988, however, Microtox test results have been less conclusive. Few recent tests have indicated sediment toxicity, and interpretation of results has been confounded by increases in bioluminescence during testing, rather than decreases. Performance standards have not been established for this test for either control or reference sediments because of difficulty in interpreting the data. The PSDDA agencies have limited use of the test to screening, pending review of the Saline Extract and other Microtox test methods. The solid phase test is currently being considered as a possible replacement for the current saline extract method for PSDDA bioassays.

Microtox Issues

Microtox appears to be sensitive to highly toxic marine sediments, and has value as a rapid-screening analytical tool for such circumstances. For sediments of low to moderate toxicity, however, results have been mixed. Problems encountered in testing include lack of response and increased luminescence. Increase in luminescence may be a response to low level toxicity in the sediment (hormesis) or to variability in the salinity of test solutions. Toxic responses have often been inconsistent with results from others in the suite of bioassays, making interpretation of test results difficult.

There continues to be considerable interest in finding a useful Microtox test method for marine sediments. Locally, EPA, the Army Corps, and PSDDA agencies are exploring Microtox options, including organizing an inter-laboratory comparison of methods. Nationally, a similar effort to evaluate the Solid Phase test is underway, sponsored by Microbics Corporation and coordinated by Dr. Phillipe Ross of The Citadel and Dr. Allen Burton of Wright State University.

PSEP Recommendations

No changes were made to the Microtox Saline Extract bioassay during the PSEP review, pending outcome of ongoing Microtox investigations.

Recommendations for SMS

Microtox is an attractive bioassay option because of its low cost and rapid turn-around. The Saline Extract test, however, appears to be less useful than originally thought for Puget Sound marine sediment testing, and limited in its utility for the SMS in its present application. A review of the SEDQUAL Microtox data may provide additional insight into optimizing usefulness of the test.

The technology for Microtox (and other rapid screening tests) continues to develop. Other Microtox tests, such as the Solid Phase and the Organic Extract tests, show promise, with greater sensitivity and consistency with results than other sediment tests in the suite. The outcome of upcoming investigations into Microtox sediment methods should be of value, possibly to suggest an alternative to Saline Extract. Unfortunately, these studies will not be completed in time for the SMS review. Fortunately, the SMS provides for Agency approval of alternative methods to accurately reflect the latest scientific knowledge. Appropriate technologies suggested by the outcome of these investigations should be evaluated for SMS use and implemented if any tests are deemed more useful than the present Microtox method.

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