

WASHINGTON STATE
DEPARTMENT OF
E C O L O G Y

Sediment Management Standards Marine Bioassays

Task II: Recommended Quality Assurance and Quality Control Deliverables

March 1996

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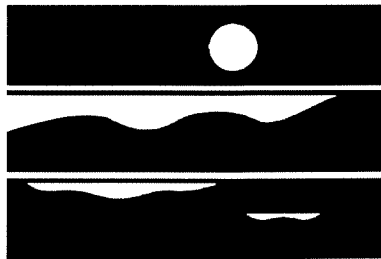
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Task II: Recommended Quality Assurance and Quality Control Deliverables

Produced for Ecology's Environmental Review / Sediment Section

by
Margaret Stinson

Environmental Investigations and Laboratory Services Program
Olympia, Washington 98504-7710

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List of Acronyms

EC ₅₀	Median Effective Concentration
EPA (U.S. EPA)	United States Environmental Protection Agency
ERSS	Environmental Review and Sediment Section
LC ₅₀	Median Lethal Concentration
PSEP	Puget Sound Estuary Program
QA/QC	Quality Assurance and Quality Control
SEDQUAL	Department of Ecology's ERSS Sediment Database
SMS	Sediment Management Standards
UV	Ultraviolet

Background

In March 1991, the Washington State Department of Ecology (Ecology) adopted a state rule, the Sediment Management Standards (SMS), Chapter 173-204 WAC, addressing sediment quality. The SMS rule was reviewed and approved by the U.S. Environmental Protection Agency Region 10, pursuant to Section 303 of the Clean Water Act. The SMS rule provides the regulatory framework for 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 and Sediment Section (ERSS) has recently completed a triennial review of the SMS as required under the authority of the Clean Water Act. Triennial review provides the opportunity for incorporating revisions and/or modifications to the SMS bioassays generated by either public comment or technical improvements. The ERSS has requested assistance from the Environmental Investigations and Laboratory Services Program in conducting this review of the SMS bioassay suite, and recommending revisions to the bioassay requirements.

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

1. Review existing SMS bioassay protocols and recommend changes if needed
2. Provide recommendations for data deliverables to be included in sediment bioassay reports submitted to Ecology
3. Identify and evaluate bioassays not presently included which should be considered for use in the SMS.

This report describes the findings of Task 2. The primary objective of Task 2 is to develop a list of recommended minimum data deliverables for bioassay monitoring reports submitted to Ecology under authority of the SMS. There are presently no defined minimum quality assurance and quality control (QA/QC) deliverables. Because it has been left to the discretion of the testing laboratories, the result has been inconsistency in the report format and type of deliverables submitted. Specified deliverables are needed to provide a consistent basis for evaluating the quality and interpreting bioassay data submitted for SMS projects

SMS Bioassays

Under the SMS rule, the potential for a sediment to cause adverse biological effects is defined by chemical criteria. Biological testing is routinely used to confirm chemical designation of sediments. These SMS chemical and biological sediment criteria are used to define:

- Marine Sediment Quality Standards, WAC 173-204-320 (3)
- Marine Sediment Impact Zone Maximum Criteria, WAC 173-204-420 (3)
- Marine Sediment Cleanup Screening Levels and Minimum Cleanup Levels, WAC 173-04-520 (3)

Four of the biological tests specified by the SMS rule are marine sediment bioassays. These tests include: the ten-day Amphipod Sediment Bioassay; any one of the Echinoderm Embryo or Bivalve Larval Bioassays; the twenty-day Juvenile Polychaete Bioassay; and the Microtox Bioassay-Saline Extract. The Amphipod and Larval Bioassays were selected to identify acute effects, based on the mortality and effective mortality endpoints, respectively. Chronic effects are evaluated using the Juvenile Polychaete growth rate endpoint and Microtox Bioassay-Saline Extract luminescence endpoint.

The use of Puget Sound Estuary Program (PSEP) Protocols is required when conducting Puget Sound Marine Sediment bioassays under the SMS (Ecology, 1995). The PSEP Protocols were developed to provide standardized methods for Puget Sound investigators, making data directly comparable and thereby appropriate for Sound-wide sediment evaluation. Descriptions of uses and limitations of toxicity endpoints are provided for each assay to allow the investigator to select endpoint(s) that will best address specific research or regulatory needs. Selection of the appropriate test species necessitates a review of potentially confounding test sediment and organism characteristics, as described by the PSEP protocols. These characteristics may include sediment grain size and salinity, as well as organism availability and spawning condition.

The PSEP Protocols have recently been revised to reflect the latest technical developments for these Marine Sediment tests. As part of the triennial review process, the SMS were amended effective December 29, 1995, to reflect those changes that will technically strengthen the regulatory process. Among these modifications to the SMS were: addition of *Strongylocentrotus drobachiensis* to the Larval species; modification of performance standards for the Larval tests; addition of *Ampelisca abdita* and *Eohaustorius estuarius* to the Amphipod suite; and adoption of a growth rate endpoint for the Juvenile Polychaete test.

Table 1 summarizes the SMS suite of marine sediment bioassays, including the SMS endpoints and reporting requirements. Performance standards and biological effects criteria for each of the SMS Marine Sediment bioassays are listed in Table 2. Changes to the SMS bioassays

resulting from the triennial review process are highlighted in Tables 1 and 2 for ease of identification.

Table 3 summarizes PSEP Protocols test conditions for the SMS suite of marine sediment bioassays. Adherence to these test conditions will optimize non-treatment conditions necessary for survival of the test organism. Reporting of all conditions observed during testing is essential for adequate review of test outcomes.

Table 1. Summary of Sediment Management Standards Marine Bioassays^{1, 2}

Bioassay	Organism	SMS Endpoint	SMS Reporting Requirements
Amphipod	<i>Rhepoxynius abronius</i>	10-day mortality	10-day mortality for each replicate; mean and standard deviation for each treatment; ³ results of hypothesis testing using one-tailed Student's t-test.
	<i>Ampelisca abdita</i>		
	<i>Eohaustorius estuarius</i>		
Larval	<i>Crassostrea gigas</i> (Pacific Oyster)	Effective mortality (combined mortality and abnormality) after 48-96 hours	Mortality and abnormality for each replicate; combined larval mortality/abnormality for each replicate; and mean and standard deviation for each treatment; ³ results of hypothesis testing using one-tailed Student's t-test.
	<i>Mytilus (edulis) galloprovincialis</i> (Blue Mussel)		
	<i>Sirongylocentrotus purpuratus</i> (Purple Sea Urchin)		
	<i>S. drobachiensis</i> (Green Sea Urchin)		
	<i>Dendraster excentricus</i> (Sand dollar)		
Juvenile Polychaete	<i>Neanthes arenaceodentata</i>	20-day mortality and growth rate	20-day mortality, biomass, and average individual growth rate for each replicate; mean and standard deviation of mortality and growth rate for each treatment; ³ results of hypothesis testing using one-tailed Student's t-test.
Microtox-Saline Extract	<i>Vibrio fischeri</i>	Luminescence after 15-minutes	Initial and final luminescence; results of hypothesis testing using one-tailed Student's t-test.

¹ Ecology, 1995.

² Shaded cells include changes to the SMS bioassays resulting from the triennial review process, with the specific change indicated in bold type.

³ A treatment includes all replicates simultaneously tested for a specific sample, control, or test/toxicant concentration.

Table 2. Performance Standards for Sediment Management Standards Marine Bioassays^{1, 2}

Bioassay	Organism	Negative Control	Reference Sediment	Biological Effects Criteria	
				Sediment Quality Standards	Sediment Impact Zones/Cleanup Levels
Amphipod	<i>Rhepoxynius abronitus</i>	Less than 10% mortality	Less than 25% mortality	Mean mortality is >25%, and is statistically different than that of the reference sediment (t-test, $p \leq 0.05$).	Mean mortality is >30% greater than that of the reference sediment, and is statistically different (t-test, $p \leq 0.05$).
	<i>Ampelisca abdita</i>				
	<i>Eohaustorius estuarius</i>				
Larval	<i>Crassostrea gigas</i> (Pacific Oyster)	Less than 30% effective mortality (combined abnormality and mortality)	The department may reject results based on unacceptably high variability. ³	Effective mortality is >15% greater than that of the reference sediment, and is statistically different (t-test, $p \leq 0.05$).	Effective mortality is >30% greater than that of the reference sediment, and is statistically different (t-test, $p \leq 0.05$).
	<i>Mytilus (edulis) galloprovincialis</i> (Blue Mussel)				
	<i>Strongylocentrotus purpuratus</i> (Purple Sea Urchin)				
	<i>S. drobachiensis</i> (Green Sea Urchin)				
Juvenile Polychaete	<i>Dendraster excentricus</i> (Sand dollar)	Less than 10% mortality; mean individual growth rate of at least 0.72 mg/day ⁴	Mean individual growth rate of at least 80% of that of the control	Mean individual growth rate is <70% of that of the reference sediment, and is statistically different (t-test, $p \leq 0.05$).	Mean individual growth rate is <50% of that of the reference sediment, and is statistically different (t-test, $p \leq 0.05$).
	<i>Neanthes arenaceodentata</i>				
Microtox-Saline Extract	<i>Vibrio fischeri</i>	Ecology will determine performance standards on a case by case basis.	Ecology will determine performance standards on a case by case basis.	Mean light output of the highest test concentration is <80% of that of the reference sediment, and is statistically different (t-test, $p \leq 0.05$).	Not applicable (N/A)

¹ Ecology, 1995.

² Shaded cells include proposed changes to the SMS bioassays resulting from the triennial review process, with the specific change indicated in bold type.

³ Ecology is currently considering standards for variability based on evaluation of in-house data.

⁴ Negative control growth rates less than 0.72 mg/day may be approved by the Department on a case-by-case basis.

Table 3. A Summary of PSEP Test Conditions for the Sediment Management Standards Marine Bioassays^{1,2}

Bioassay	Organism	Duration	Temp. (°C)	Organisms/Replicates	Test Volumes	Renewal	Feeding	Aeration	Lighting/Photoperiod	Dilution Water
Amphipod	<i>Rhepoxynius abronius</i>	10-days	15 ± 1	Twenty organisms in each of five replicates	175 mL (2 cm sediment, in water to 950 mL, in 1-Liter beaker	None	Not fed	Required throughout testing	Normal room lighting/continuous	Filtered (0.45 micron) or UV sterilized; held no longer than 2-days at test temperature before use; salinity adjusted to 28 ± 1 ppt (to ambient interstitial salinity for <i>E. estuarius</i>)
	<i>Ampelisca abdita</i>		20 ± 1							
	<i>Eohaustorius estuarius</i>		15 ± 1							
Larval	<i>Crassostrea gigas</i> (Pacific Oyster)	48-hr	20 ± 1	20,000 - 40,000 organisms in each of five replicates	18 gm sediment, in 900 mL water, in 1-Liter beaker	None	Not fed	Required if dissolved oxygen < 60% saturation or if ammonia or sulfides are present	Normal room lighting/14-hr light 10-hr dark	
	<i>Mytilus (edulis) galloprovincialis</i> (Blue Mussel)		16 ± 1							
	<i>Strongylocentrotus purpuratus</i> (Purple Sea Urchin)		15 ± 1							
Juvenile Polychaete	<i>S. drobachensis</i> (Green Sea Urchin)	20-days	20 ± 1	Five organisms in each of five replicates	175 mL (2 cm sediment, in water to 950 mL, in 1-Liter beaker	One-third overlying water renewed every third day	Fed 40 mg Tetra-Marin per replicate every other day	Required throughout testing	Ambient, low to moderate/continuous	Treated as described above; salinity adjusted to 28 ± 2 ppt
	<i>Dendraster excentricus</i> (Sand dollar)									
	<i>Neanthes arenaceolentata</i>									
Microtox-Saline Extract	<i>Vibrio fischeri</i>	15-min	15 ± 1	10 uL rehydrated bacterial suspension per cuvette	Supernatant from 30 gm sediment and 10 mL diluent	N/A	N/A	N/A	N/A	Microtox diluent

¹PSEP, 1995.

²These are provided only as guidelines for optimal performance of these tests. Each individual protocol should be reviewed for additional conditions unique to the test, organism, or specific needs of the study.

Reporting Requirements

SMS bioassay reports will be prepared in such a way as to establish whether procedures and test conditions produced results acceptable for evaluation against SMS biological effects criteria. Methods, materials, and results should be clearly described. Any deviations from standard procedures should be noted, along with the analyst's evaluation of how data quality might be impacted. At a **minimum** the report should include:

- Summary of procedures
- Quality assurance/quality control
- Results
- Analyst's narrative evaluation of testing
- Detailed documentation of methods and results

The recommended reporting format is shown on the following pages. Original documentation pertinent to the conduct and findings of the test should be kept on file by the Analytical Laboratory for a period not less than ten years, WAC 173-204-610 (4).

In addition to the hard copy report, data submittal in electronic format compatible with Ecology's SEDQUAL software is recommended. The SEDQUAL format has been designed to facilitate QA review of sediment test data, and to transfer sediment quality data into the Ecology database. Electronic data submittal has so expedited processing of permit requests that it is recommended the SMS Rule be modified to require electronic submission of all sediment data.

Examples of the SEDQUAL format are in Appendices I through V. Templates included are:

Appendix I:	Station Description Template
Appendix II:	Survey Description Template
Appendix III:	Sediment Sample Template
Appendix IV:	Bioassay Data Entry Template
Appendix V:	Bioassay Control Data Entry Template

The electronic format for SEDQUAL data submittal may be obtained from Tuan Vu at (360) 407-7449.

Recommended Reporting Format and Deliverables

The preferred format for data reporting is in two parts: the Case Summary and Supporting Documentation.

The purpose of a Case Summary is to provide a complete and concise overview of test procedures, outcome, and the analyst's review of the significance and quality of the test outcome. A reviewer should be able to quickly summarize and understand the test's significance relative to the study objectives.

The second section, Supporting Documentation, provides all the background information necessary to support the information presented in the Case Summary. It should include all raw data, bench sheets, statistical analyses and quality assurance information.

A recommended reporting format and list of deliverables follow.

Case Summary

The following are recommended for inclusion in the Case Summary:

1. Project/sample(s) identification
2. Test method
 - Species tested
 - Protocol reference, description of any nonstandard procedures
 - Analytical Laboratory responsible for analyses
3. Case Narrative - analyst's evaluation of the test(s), including
 - Deviations from protocol, problems noted during testing
 - Effect of deviation or problems on utility (quality) of data
 - Concurrent testing of sample, reference, and control sediments
 - Re-analyses required, which samples, and reason(s) for
 - Negative control sediments in compliance with SMS performance standards for test validation
 - Reference sediments in compliance with SMS performance standards for test validation
 - Written documentation of real time communications.

4. Tabulated summary of test response replicate mean and standard deviation (s.d.) for each of the following (Figure 1)
 - Negative control sediment
 - Reference sediment
 - Sample sediments
 - Seawater control (for larval test)
5. Outcome of statistical analyses
 - Summary of statistical methods and software programs used
 - Use of control and reference sediment responses in statistical analyses
 - Designation of which sample responses were statistically significant
6. Positive control response
 - Reference toxicant used; source; details of test method
 - Measured or nominal concentrations tested
 - Estimate of LC₅₀ and/or EC₅₀
 - Comparison to laboratory reference toxicant control chart from previous tests (within 2 s.d. of the control chart mean)
7. Data submittal in electronic format, compatible with Washington State Department of Ecology SEDQUAL software.

Supporting Documentation

The following are recommended for inclusion as Supporting Documentation:

1. Identification for each control, reference and sample sediment
 - Source (geographic location and latitude/longitude)
 - Collection date and time
 - Sampling device and depth
 - Physical description (appearance)
 - Holding conditions (4°C, darkness, ≤2 weeks)
 - Date, condition received at Laboratory
 - Chain-of-custody documentation

Case Title:
Batch Identification:
Date Tested:

	Station	Sample Number	Mean Test Response (s.d.)*
Control Sediment			
Seawater Control			
Reference Sediment			
Test Sediment			
Reference Toxicant			

*Statistically significant relative to reference sediment response (Student's t-test, $p \leq 0.05$).

Figure 1. Suggested Format for Test Response Summary¹

¹This prototype shows a brief, but complete, overview of test results and significant responses, providing a consistent format to facilitate data review.

2. Test method
 - Dates and times of test initiation and termination
 - Sample preparation and handling procedures
 - Test conditions - duration, temperature, lighting/photoperiod, test vessels/volume/replication, organisms per replicate, renewal, feeding, aeration
 - Dilution water source, collection date, treatment (filtration, UV, etc.), details of salinity adjustment
 - Test organisms - source (purchased or field collected), date, lifestage/age/condition, confirmation of taxonomic identification, acclimation
 - Frequency and type of observations made during testing
 - Endpoint(s) measured

3. Test results - raw data in tabular form and bench sheets
 - Test initiation and termination
 - Daily observations made during testing
 - Measurement of water quality parameters (dissolved oxygen, pH, salinity, ammonia, and sulfides), and methods used for measurements
 - Measurement of bulk parameters (ammonia, grain size distribution, interstitial salinity, sulfides, total organic carbon), and methods used for measurements

4. Statistical analyses
 - Methods used, including specific formulae, and software programs used for analyses
 - Printouts

5. Positive control
 - Dates and times of test initiation and termination
 - Raw data for reference toxicant test
 - Method used for estimate of LC_{50} and/or EC_{50}
 - Copies of printouts for LC_{50}/EC_{50} analyses

References

- Betts, Brett. 1995. Amphipod Bioassay: Modifications to the Sediment Management Standards (SMS) Amphipod Test Protocol and Proposed Modifications to the SMS Rule. Issue paper prepared for the 1995 SMS Triennial Review.
- Betts, Brett. 1995a. Neanthes 20-Day Bioassay: Modifications to the Sediment Management Standards (SMS) Neanthes Test Protocol and Proposed Modifications to the SMS Rule. Issue paper prepared for the 1995 SMS Triennial Review.
- Ecology. 1995. Washington State Department of Ecology. Sediment Management Standards. Chapter 173-204 WAC. December 1995.
- PSEP. 1995. Puget Sound Estuary Program. Recommended Guidelines for Conducting Laboratory Bioassays of Puget Sound Sediments. Prepared for U.S. EPA Region 10, Office of Puget Sound, by Puget Sound Water Quality Authority. Revised July 1995.
- Sparks-McConkey, Pamela. 1995. Modification to the Sediment Management (SMS) Larval Test Protocol and Associated Proposed Modifications to the SMS Rule. Clarification paper prepared for the 1995 SMS Triennial Review.

Appendices

#	Department of Ecology - Sediment Management Unit																				
#	SEDQUAL Database																				
#	STATION DESCRIPTION TEMPLATE																				
#	Asterisks indicate ** REQUIRED ** fields.																				
#	Empty cells MUST contain a # sign.																				
#	ENTER PROJECT INFORMATION TEXT HERE, FOR EXAMPLE:																				
#	PIER D SUPPLEMENTAL SAMPLING																				
#	8/10/93																				
#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18			
#	*	*	*	*	*	*	*	*	*	SPCN	SPCE	*	SPCZone				*				
	STATION-ID	STATION TYPE	LAT-DEG	LAT-MIN	LAT-SEC	LON-DEG	LON-MIN	LON-SEC	STATE PLANE	STATE PLANE	STATE COORDINATE	STATE	STATE PLANE	COORN (DEPT)	COORN (NEW FIELD)	GEO CODE	BASIN CODE	SUB BASIN			
#		ST-SUB TIDAL	IT-INTER TIDAL						COORN NORTH	COORN EAST	ZONE										
EXAMPLE:																					
PIER-D93	X1	ST	47	34	12	###	11	29	#	#	WA	N	#	#	#	#	#	CPSIDY			

Appendix I. Station Description Template

#	Department of Ecology - Sediment Management Unit										
#	SEDQUAL Database										
#											
#	SURVEY DESCRIPTION TEMPLATE										
#	Asterisks indicate ** REQUIRED ** fields.										
#	Empty cells MUST contain a # sign.										
#	ENTER PROJECT INFORMATION TEXT HERE, FOR EXAMPLE:										
#	Side Slope Verification Sampling Report.										
#	Prepared for Port of Tacoma by HartCrowser J-3875-03 June 15, 1994.										
#		1	2	3	4	5	6	7	8		
#											
#	SURVEY DESCRIPTION #										
			STATION NAME	BEG DATE	END DATE	AGENCY	CHIEF SCIENTIST	PROGRAM	PROJECT		
8 CHARACTERS	40 CHARACTERS			(COLLECTION)							
EXAMPLE:											
PSMON_94	1994 PUGET SOUND MONITORING REPORT		6/15/94	6/18/94	DOE	JO COOL					

Appendix II. Survey Description Template

#	Department of Ecology - Sediment Management Unit		DATE	SAMPLID	FIELD REP	SUBSAMPLE	BIOS_TYPE	BIOS_VAR	NODC_CODE	ANAL_GP	DILUTION	LAB REP	INITIAL_VAL	FINAL_VAL	BSSY_UNITS	ASSAY_TRTM
	SEDQUAL_Database															
#	BIOASSAY DATA ENTRY TEMPLATE															
#	USE # TO INDICATE WHERE DATA IS NOT AVAILABLE															
#	Asterisks indicate ** REQUIRED ** fields.															
#	ENTER PROJECT INFORMATION TEXT HERE, FOR EXAMPLE:															
#	Side Slope Verificati WEST BLAIR TERMINAL DEVELOPMENT															
#	Prepared for Port of Tacoma by HartCrowser J-3875-03 June 15, 1994.															
#	SURVEY	STN ID														
#	CHARACT-SUB	INMDDYY														
#	IT-INTER TIDAL					AMP10	ABNM									
#	BR-BRACKISH					BIVLV	BIOM									
#	LE-FRESHWATER LENTIC					ECHIN	EMERG									
#	LO-FRESHWATER LOTIC					MICTX	LUM									
#						NEANT	MORT									

Appendix IV. Bioassay Data Entry Template

Department of Ecology - Sediment Management Unit		SEDQUAL Database		BIOASSAY CONTROL DATA ENTRY TEMPLATE		USE # TO INDICATE WHERE DATA IS NOT AVAILABLE		Asterisks indicate ** REQUIRED ** fields.		ENTER PROJECT INFORMATION TEXT HERE, FOR EXAMPLE:		EXACT CITATION:		Side Slope Verification Sample WEST BLAIR TERMINAL DEVELOPMENT		Prepared for Port of Tacoma by HartCrowzer J-3875-03 June 15, 1994.	
SURVEY	BIOASS_TYPE	BIOASS_VAR	NODC_CODE	ANAL_GRP	DILUTION	NEG_CTRL	LAB_REP	INIT_VALUE	FINAL_VALUE	BSSY_UNIT	ASSY_TRIM						
8 CHARA	AMP10-AMH	ABNM-ABNM	ABNOMALITY OR BATCH	percent	Y=YES N=NO			# of animals g	# of animals g	IND-Individual LUM=Lumin MGD=Millig PCT=Percent	D-Dionized water extract O-Organic extraction S- Saline extraction						
	BIVLV-BIVA	BIOM-BIOMASS															
	ECHIN-ECHI	EMER-EMERGENCE															
	MICTX-MIC	LUM-LUMINOSITY															
	NEANT-NEA	MORT-MORTALITY															

Appendix V. Bioassay Control Data Entry Template