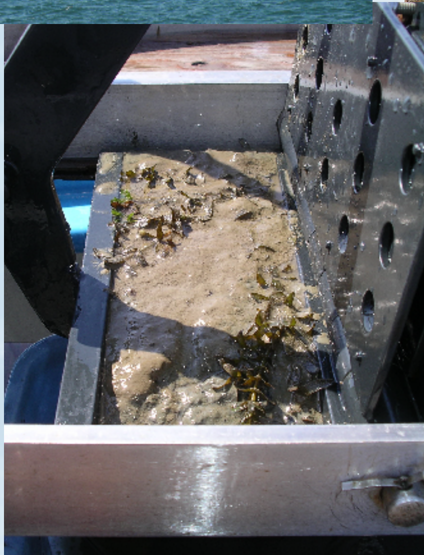
Sediment Quality Assessment Technical Support Manual



Steven M. Bay
Darrin J. Greenstein
Ashley Parks
David Gillett
Wayne Lao
Dario W. Diehl

Southern California Coastal Water Research Project

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Steven M. Bay, Darrin J. Greenstein, Ashley Parks, David Gillett, Wayne Lao, and Dario W. Diehl

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PREFACE

The incorporation of sediment quality objectives into the State Water Board's water quality policy represents a major development in the application of sediment quality assessment for regulatory programs. Previously, the methods and data interpretation process for sediment monitoring data has been variable due to the lack of statewide objectives and variable data interpretation methods. The goal of this document is to help organizations make the transition to the new or revised methods specified in the new policy by providing information on methods and data interpretation.

This manual has been developed to assist organizations in implementing the new methods. While much effort has been devoted to checking the information for accuracy, this manual may undergo revision because of feedback from other organizations. The reader is encouraged to check the Water Board's sediment quality objectives web page and the SCCWRP sediment quality assessment web pages for future revisions to this document.

This information in this document is intended to provide end users with guidance for application of the assessment tools for protection of aquatic life described in the State Water Board's Water Quality Control Plan for Enclosed Bays and Estuaries - Part 1 Sediment Quality. This third edition of the technical support manual includes updates of the Aquatic Life SQO assessment framework and the addition of tools and recommendations for the Human Health SQO assessment framework, as adopted by Water Board actions in 2018. This document consists of recommended approaches and does not represent regulation or direction from the State Water Board.

LIST OF ACRONYMS

AET	Apparent Effects Threshold
ALSQO	Aquatic Life Sediment Quality Objective
AMBI	AZTI Marine Biotic Index
ASE	Accelerated Solvent Extraction
ATL	Advisory Tissue Level
BPTCP	Bay Protection and Toxic Cleanup Program
BRI	Benthic Response Index
BSAF	Biota-Sediment Accumulation Factor
CA LRM	California Logistic Regression Model
CASQO	California Sediment Quality Objectives
CDFW	California Department of Fish and Wildlife
COC	Chain of Custody
CRM	Certified Reference Material
CSI	Chemical Score Index
CSM	Conceptual Site Model
DCM	Dichloromethane
DI	De-ionized
DQO	Data Quality Objective
DNQ	Data Not Quantifiable
DST	Decision Support Tool
EC50	Effective Concentration, half maximal
EMAP	Environmental Monitoring and Assessment Program
EPA	Environmental Protection Agency
FCG	Fish Contaminant Goal
GC-ECD	Gas Chromatography-Electron Capture Detector
GC-MS	Gas Chromatography-Mass Spectrometry
GIS	Geographical Information System
GPS	Global Positioning System
HDPE	High Density Polyethylene
HHSQO	Human Health Sediment Quality Objective
HPAH	High Molecular Weight Polycyclic Aromatic Hydrocarbon
HR	Home Range
IBI	Index of Biotic Integrity
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICZN	International Commission on Zoological Nomenclature
IUPAC	International Union of Pure and Applied Chemistry
LDPE	Low Density Polyethylene
LOE	Line of Evidence
LPAH	Low Molecular Weight Polycyclic Aromatic Hydrocarbon
M-AMBI	Multivariate AMBI
MCS	Monte Carlo Simulation
MDL	Method Detection Limit
MLOE	Multiple Lines of Evidence
MSD	Minimum Significant Difference
ND	Non-Detect

NIT	Negative Indicator Taxa
NOAA	National Oceanic and Atmospheric Administration
O/E	Observed/Expected
OEHHA	Office of Environmental Health Hazard Assessment
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
PDMS	Polydimethylsiloxane
PE	Polyethylene
PED	Polyethylene Device
PIT	Positive Indicator Taxa
PNA	Percent Normal-Alive
ppb	parts per billion
ppt	parts per thousand
PRC	Performance Reference Compound
PSD	Passive Sampling Device
PTFE	Polytetrafluroethane
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
QC	Quality Control
RBI	Relative Benthic Index
RIVPACS	River Invertebrate Prediction and Classification System
RL	Reporting Limit
RMP	Regional Monitoring Program for Water Quality in the San Francisco Estuary
RPD	Relative Percent Difference
SAS	Statistical Analysis System
SCAMIT	Southern California Association of Marine Invertebrate Taxonomists
SCCWRP	Southern California Coastal Water Research Project
SWAMP	Surface Water Ambient Monitoring Program
SIM	Selected Ion Monitoring
SOP	Standard Operating Procedure
SQGs	Sediment Quality Guidelines
SQO	Sediment Quality Objective
SPMD	Semipermeable Membrane Device
SPME	Solid Phase Microextraction
SRM	Standard Reference Material
SWI	Sediment-Water Interface
SWRCB	State Water Resources Control Board
TIE	Toxicity Identification Evaluation
TOC	Total Organic Carbon
TMDL	Total Maximum Daily Loads
TWV	Taxa Richness Weighted Value
UCL	Upper Confidence Limit
USEPA	United States Environmental Protection Agency

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SECTION I: BACKGROUND AND CONCEPTUAL APPROACH

Background

Sediment quality influences the overall condition of a water body. Sediments act as a reservoir for contaminants that can be transferred to the water column and are also a primary source of contaminant exposure for sediment-dwelling organisms. Sediment quality assessment has been an important feature of many California monitoring programs. It was a major focus in the Bay Protection and Toxic Cleanup Program (BPTCP; (Anderson *et al.* 1997)), the California Environmental Monitoring and Assessment Program (EMAP; (USEPA 2005a)), the Regional Monitoring Program for Water Quality in the San Francisco Estuary (SFEI 2005), and the Southern California Bight Regional Monitoring Program (SCCWRP 2003, 2007; Southern California Bight 2008 Regional Monitoring Program Coastal Ecology Committee 2012).

Sediment is a complex matrix of components and forms. Consequently, evaluating sediment quality based on a single type of data (line of evidence) is problematic. For example, bulk measures of chemical concentration fail to differentiate between the fraction of a contaminant that is tightly bound to sediment and that which is biologically available. Multiple mechanisms of contaminant exposure, including uptake of chemicals from interstitial water, sediment ingestion, and bioaccumulation through the food web further complicate interpretation of sediment chemistry data. For these reasons, sediment quality assessment often involves simultaneously evaluating multiple lines of evidence (MLOE) that measure both contaminant exposure and effects on organisms: an approach commonly known as the sediment quality triad (Long and Chapman 1985). Lines of evidence (LOEs), such as sediment chemistry, toxicity, and benthic community condition are often used. Virtually all of the ambient sediment quality monitoring programs in this country rely on more than one line of evidence (USEPA 1998; Crane *et al.* 2002; MacDonald and Ingersoll 2002; USEPA 2004). Such programs include the two largest nationwide estuarine monitoring programs: the United States Environmental Protection Agency (USEPA) EMAP and the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends Program, as well as California's BPTCP (Anderson *et al.* 1997; Fairey *et al.* 1998; Phillips *et al.* 1998; Anderson *et al.* 2001; Hunt *et al.* 2001) and the Southern California Bight Regional Monitoring Program (Schiff *et al.* 2016).

In 2003, the California State Water Resources Control Board (SWRCB) initiated a program to develop sediment quality objectives (SQO) for chemical contaminants in bays and estuaries based on an MLOE approach. This first phase of the California SQO (CASQO) program was completed in 2008, which resulted in the SWRCB's adoption of new policy regarding sediment quality as part of the water quality control plan for enclosed bays and estuaries (SWRCB 2008). This policy contains two narrative sediment quality objectives: one for the protection of aquatic life due to the direct effects of exposure to sediment contaminants and one for the protection of human health from indirect effects through the consumption of seafood. Assessment frameworks to evaluate sediment for attainment of these SQOs were adopted by the Water Board and approved by the EPA in 2009 (Aquatic Life SQO) and 2018 (Human Health SQO).

While the SQO policy specifies the types of measurements and describes how to interpret the results, many technical details regarding the methodologies are referenced in other documents. As a result, new users of the CASQO assessment approaches may have difficulty obtaining the information necessary to apply the tools correctly. The objective of this document is to describe

these technical details in an integrated manner to facilitate the assessment of sediment quality using the CASQO approach.

Scope of the Manual

This document was prepared by the State Water Board's technical team to provide end users with guidance for application of the assessment tools identified in the State Water Board's Water Quality Control Plan for Enclosed Bays and Estuaries - Part 1 Sediment Quality (referred to as SQO policy in this document). This document consists of recommended approaches and does not represent regulation or direction from the State Water Board.

This manual describes the methods and provides recommendations for obtaining sediment quality data necessary to apply the SQOs for aquatic life and human health protection. This manual does not provide information for evaluating sediment quality with respect to the narrative SQO for the protection of fish and wildlife.

The information presented in this document is targeted towards the technician or scientist responsible for generating or analyzing the data and assumes a basic familiarity with the types of measurements described. It is also intended to serve as a reference for environmental managers in the design and interpretation of monitoring studies.

This manual is intended to supplement current standard methodologies applied in California, rather than providing comprehensive instructions for each type of analysis. As such, the different chapters contain varying levels of detail about sediment assessment procedures based on the amount of information that is already available elsewhere. If methods are published in other documents (as is the case for the sediment chemistry analyses), they are referenced in the text so that the reader may acquire them separately. If no other comprehensive sources for the methods exist (as is the case for some of the methodology described for sediment toxicity and benthic community composition assessment), detailed information is included in this manual. The manual also provides step-by-step instructions and examples for integrating the various data types to result in an assessment of sediment quality that is consistent with the CASQO assessment framework.

This manual is intended solely to assist end users with making an accurate assessment of sediment quality; it does not provide guidance for how to use the information in a regulatory context. Information on the use of the assessment information in monitoring and regulatory applications is provided in the SQO policy and decisions regarding the use of this information are the responsibility of the regulatory and monitoring agencies involved in the program.

Following the guidance in this manual will generate a high-quality set of integrated data that will be valuable for multiple current and future uses, such as evaluating regional sediment quality and assessing attainment of regulatory program objectives. To attain the full benefit of the time and resources invested in obtaining these results, it is essential that robust data management and quality assurance programs are used to make these data accessible to end users and the public. While data management recommendations specific to certain data types are included in some of the following chapters, there are several recommendations that are applicable to most elements of the assessment:

- Raw data should be submitted and archived in statewide and regional databases that use a consistent format and provide public access. Data, especially those from State-funded or regulatory programs, should be submitted to the California Data Exchange Network (CEDEN). This will help create a long-lived historical record and facilitate use of the data for statewide assessments such as listing regions that fail to meet water quality standards under the federal Clean Water Act section 303(d). Data should also be included in regional databases appropriate for the study type and location. Examples of these databases include the Surface Water Ambient Monitoring Program (SWAMP), the Southern California Bight Regional Monitoring Program, and Regional Monitoring Program for Water Quality in the San Francisco Estuary (RMP).

- Technical reports for sediment quality assessments should include the raw data in tabular format as appendices and supplemental digital files. These data should be sufficiently complete so that published results can be independently verified. These reports should include contact information for persons qualified to respond to questions regarding data access and provide program metadata.

- Technical report supplemental data files should be made available in a format compatible with commonly used statistical analysis programs, such as those available for R and Python (e.g., xlsx, csv, tab-delimited txt).

- Links to specialized data analysis tools used in the study should be provided, including links to the organization's software repository (e.g., GitHub page) so that users can access the latest version of the resource. Similarly, GIS layers used in the analysis should be made available so that others can reproduce maps used in published reports.

- Information on data quality should be included with the analysis results, such as a description of the quality assurance project plan (QAPP) guiding data collection and tables summarizing the level of attainment of data quality objectives (DQOs).

The CASQO assessment frameworks were developed for assessing attainment of beneficial uses in California enclosed bays and estuaries. While the overall conceptual approach and many of the measurements are appropriate for other habitats, many of the indices and response ranges used to interpret the data have been calibrated to specific habitats and should not be applied to other areas (e.g., offshore waters and freshwater habitats) without additional development and validation.

Chapter 1: Conceptual Approaches

Conceptual Approach for Aquatic Life SQO Assessment

Implementation of the Aquatic Life SQO (ALSQO) requires a series of specific analyses and a data interpretation framework based on the integration of three Lines of Evidence (LOEs): 1) sediment chemistry, 2) sediment toxicity, and 3) benthic community (Figure 1.1).

A variety of environmental conditions exist within bays and estuaries that limit the scope of application for some of the tools, especially benthic community indices. Benthic species composition and abundances vary naturally from habitat to habitat and expectations for reference condition and measurements of deviation from reference should vary accordingly. Most of the benthic indices described in this manual are only applicable for certain euhaline and polyhaline habitats. A different index for evaluating benthic community composition in other bay and estuarine habitats should be used. Careful attention should be paid to verify that the appropriate benthic indices are used for the habitat of interest. The same tools for evaluation of sediment chemistry and sediment toxicity can generally be used among the different habitats. However, an alternate toxicity test may need to be used in habitats where the salinity levels are below the tolerance range of the toxicity test species specified for the euhaline and polyhaline habitats.

Samples are optimally collected during a summer index period from June to September. Physical environments in many enclosed bays are stable and most similar from year to year in the summer. Benthic community composition and abundances have similar stability patterns; measurement of benthic community disturbance is therefore most reliable when sampling is conducted in summer. Sediment samples for each type of analysis (i.e., toxicity, chemistry, and benthos) should be collected at the same time, to minimize variability associated with station positioning or seasonal events.

The ALSQO data integration framework described in this document (Figure 1.1) requires that all three LOEs are measured according to the methods specified. While each LOE provides useful information and can be measured independently, all three LOEs are needed to provide a more accurate and reliable measure of sediment quality. Integration of the three LOEs produces a categorical assessment for each station that indicates the potential for contaminant-related impacts to the benthic community. These categories range from Unimpacted (best sediment quality) to Clearly Impacted (greatest severity of impact and confidence in the assessment).

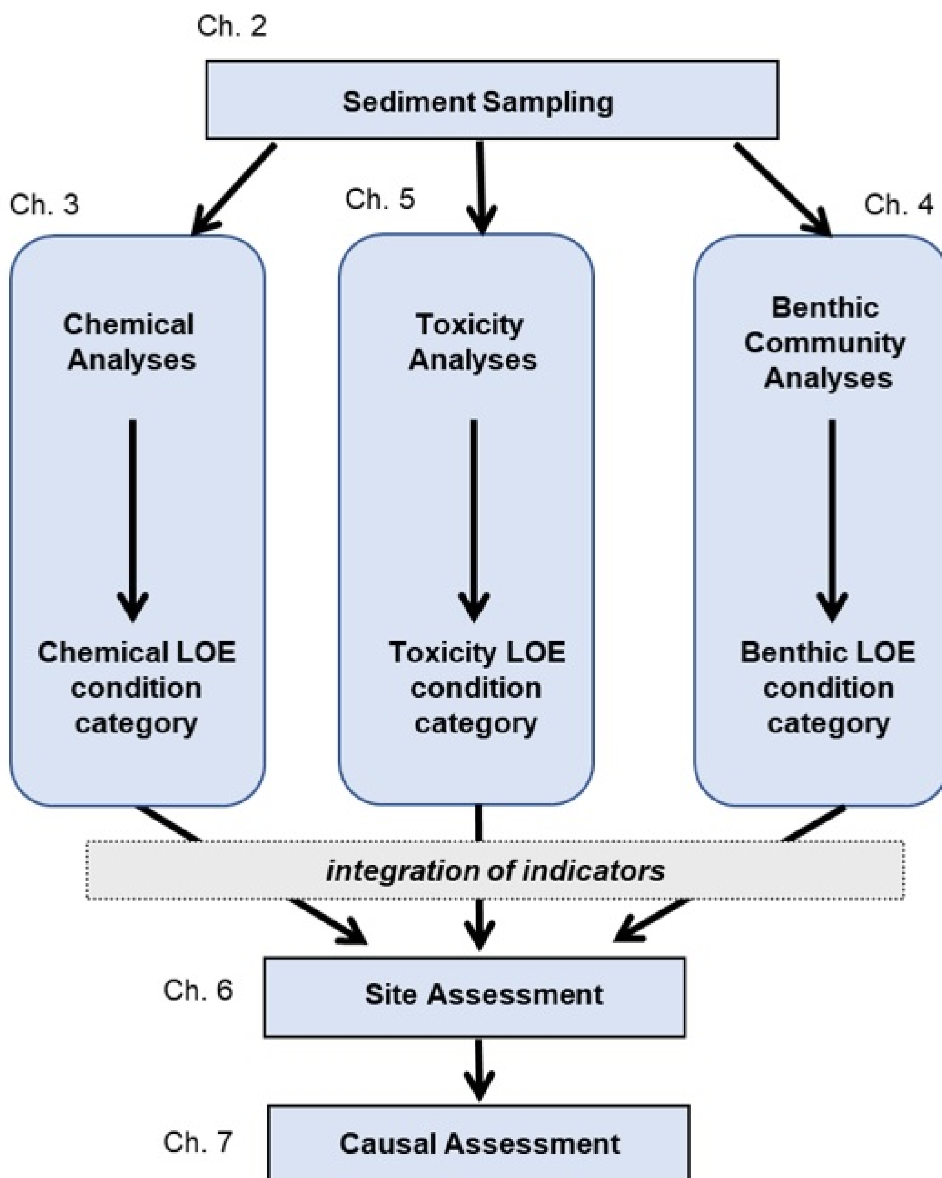


Figure 1.1. Overview of the Aquatic Life SQO station assessment process.

Conceptual Approach for Human Health SQO Assessment

Two indicators are evaluated to assess the Human Health SQO (HHSQO): 1) **Chemical Exposure**, defined as the extent to which pollutant concentrations in seafood pose unacceptable health risks to human consumers, and 2) **Site Linkage**, defined as the relative contribution of sediment contamination at the site to seafood contamination (Figure 1.2). Chemical exposure is evaluated by comparison of fish tissue contaminant concentrations to seafood consumption advisory levels established by California. Site linkage describes the strength of the association between sediment contamination (including flux into the water column) and seafood contamination. The presence of a strong linkage with site sediment is a critical element in determining whether the HHSQO is attained for the site because it indicates whether health risks are likely due to site conditions (relevant to the SQO) as opposed to off-site factors (e.g., fish movement or watershed loading) that are the focus of other regulatory programs. The degree of site linkage indicates the relative bioaccumulation due to sediment contamination from the site which is estimated using bioaccumulation models.

Integration of the chemical exposure and site linkage indicators produces a categorical site assessment. The categories represent the magnitude of health risk associated with sediment contamination within the site. The site assessment category indicates whether the human health SQO is met at the site (e.g., impacted by sediment contamination). These categories range from Unimpacted (best sediment quality) to Clearly Impacted (greatest deviation from the protected condition described in the HHSQO) and are structured similarly to the categories used to assess ALSQO.

Application of the assessment framework is organized into three tiers (Figure 1.3). Each tier represents an increasing level of effort and complexity to enable the assessment to match variations in data availability, site complexity, and study objectives.

Tier 1 consists of a screening assessment of tissue and/or sediment chemistry data to determine whether there is sufficient potential concern for human health impacts to warrant a complete site assessment. The purpose of Tier 1 is to provide an option for initial site evaluation with relatively low data requirements, thereby enabling rapid identification of areas of low concern (USEPA and U. S. Army Corps of Engineers 1991, 1998; Hope 2009). Sediment or tissue chemical concentration data are interpreted using standardized conservative assumptions to evaluate concern for human consumers of seafood. If Tier 1 results indicate a potential concern, the analysis proceeds to Tier 2; otherwise, the site is determined to meet the SQO and further assessment is not needed.

Tier 2 represents a complete and standardized site assessment. Both tissue and sediment chemistry data, along with additional site-specific information, are evaluated to determine human health risk. Tier 2 differs from Tier 1 in three important respects. First, some default Tier 1 assumptions and parameters are replaced with site-specific assumptions and parameters, such as seafood forage area, and habitat characteristics. Second, estimates of chemical exposure (from tissue data) and site linkage (from sediment data) are compared to classify the site condition. Finally, the Tier 2 analysis produces a probabilistic output of site linkage to help communicate data variability and uncertainty. If Tier 2 results indicate an acceptable condition, the sediment is classified as meeting the HHSQO.

The Tier 3 assessment may be employed when trigger criteria are met and the Tier 2 results are deemed unreliable due to site-specific conditions such as other sources of contamination, temporal variability, or substantial uncertainty in exposure parameters (e.g., seafood exposure to site sediments, site physical constraints (e.g., small size), food web structure, contaminant gradients, different human consumption rate, or bioavailability of contaminants in site sediments). The specifics of the Tier 3 assessment method are determined on a site-specific basis and might include the collection of additional data or use of alternative data analysis methods. However, final interpretation of the data to determine site conditions follows the same steps as in Tier 2.

The HHSQO assessment approach focuses on the most important processes that govern the indirect effects of sediment contamination to human consumers of seafood. It is simpler than some sediment risk assessment models (e.g., Bridges *et al.* 2005). This model does not address other potential factors that could be important for individual sites. A site-specific Conceptual Site Model (CSM) is needed to address factors such as contaminant transfer between deep sediment and surface sediment; toxicological effects on aquatic plants or invertebrates; or changes over time in contaminant concentrations and transfer pathways. These factors may be important in site-specific evaluations (Davis 2004, Bridges *et al.* 2005, Gobas and Arnot 2005, Greenfield and Davis 2005) and merit use of a Tier 3 assessment. Often these issues are considered when management action or remedial alternatives are being considered, which is outside the scope of the HHSQO assessment. Use of different analytical and modeling approaches may be needed to develop management actions.

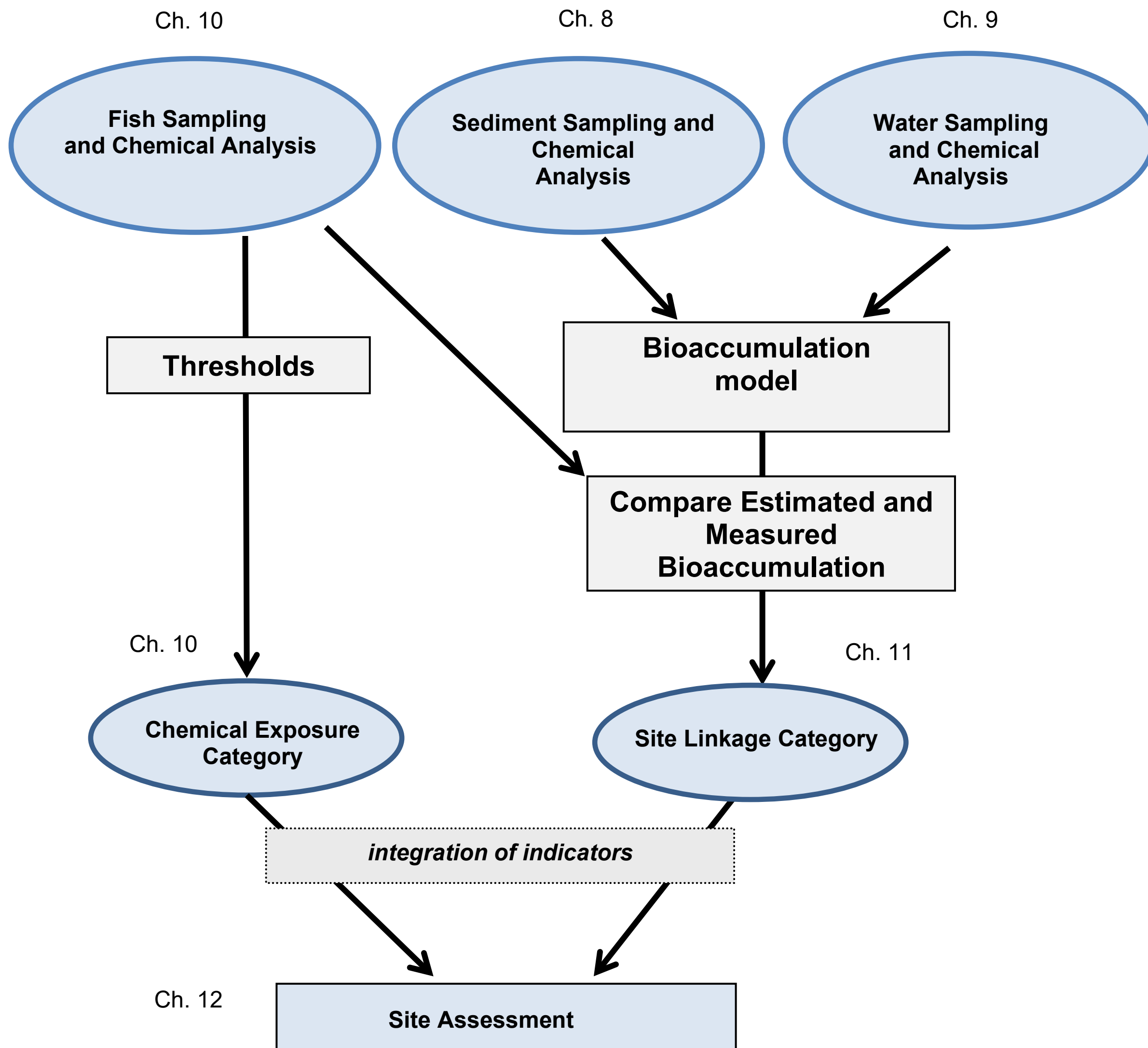


Figure 1.2. Overview of the Human Health SQO site assessment process.

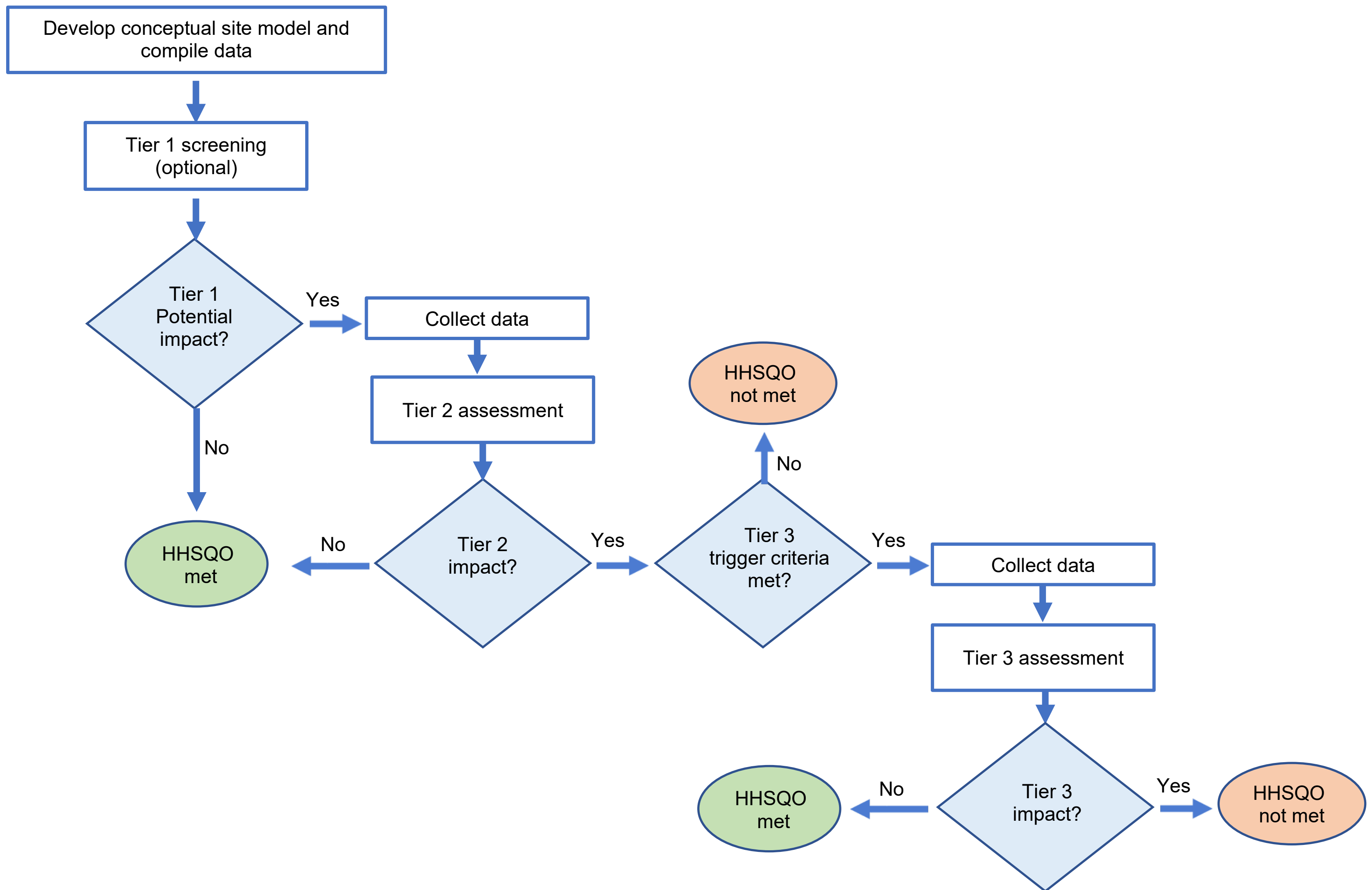


Figure 1.3. Tiered HHSQO assessment process.

Organization of the Manual

This manual is organized into sections and chapters addressing the key components of each SQO assessment framework (Figures 1.1 and 1.2).

Section I provides recommendations and describes methods for testing, data analysis, and interpretation for the ALSQO assessment.

- Chapter 2 provides recommendations for **sediment sampling.**
- Chapter 3 provides recommendations for sediment chemistry analysis and shows the steps in deriving the **chemistry line of evidence result (Chemistry LOE).**
- Chapter 4 provides recommendations for assessment of the benthic community and shows the steps in deriving the **benthic community line of evidence result (Benthic LOE).**
- Chapter 5 provides recommendations for sediment toxicity analysis and shows the steps in deriving the **toxicity line of evidence result (Toxicity LOE).**
- Chapter 6 describes how to integrate the three LOEs to determine an overall **station assessment** for different types of habitats.
- Chapter 7 describes approaches for **causal assessment**, the process of determining the cause of impacts.

Section II provides recommendations and describes methods for testing, data analysis, and interpretation for the HHSQO Assessment.

- Chapter 8 describes special considerations for **sediment sampling** to support use of the HHSQO assessment framework.
- Chapter 9 provides recommendations and methods for **water sampling and analysis** for dissolved organochlorine compounds.
- Chapter 10 provides recommendations for **fish sampling, tissue chemical analysis and calculation of the Chemical Exposure indicator.**
- Chapter 11 describes the use of bioaccumulation modeling for determining the **site linkage indicator.**
- Chapter 12 describes integration of the data to determine an overall **site assessment.**

SECTION II: AQUATIC LIFE SQO ASSESSMENT

Chapter 2: Sediment Sampling

Objectives

Analyses of benthic community condition, chemistry, and toxicity require the collection of samples of surface sediment that are representative of in situ conditions and free from sampling artifacts such as degradation or contamination. Each type of analysis has unique requirements for sample collection, onboard processing, and storage. The objectives of this chapter are to provide an overview of the key elements of field sampling in subtidal marine and estuarine habitats.

Scope

This chapter is intended to supplement current sediment sampling protocols used in California for enclosed bays and estuaries by providing recommendations for methodology suitable for application within the CASQO assessment framework. It covers a wide range of sampling activities including a discussion of common grab samplers, a summary of sample handling procedures to prepare for eventual laboratory analyses, and a description of recommended approaches for ensuring sample quality and integrity for each type of analysis.

When and Where to Sample Sediments

Samples are optimally collected during summer months from July to September. Physical environments and benthic community characteristics are relatively stable and most similar from year to year in the summer. This is especially true in areas where rainfall and freshwater influence are high, such as San Francisco Bay and Northern California.

Complete sets of tools for assessing sediment quality in the CASQO program are available only for two of California's six enclosed bay habitats: Southern California Marine Bays, and San Francisco Bay Polyhaline. This limitation is primarily based on the lack of a full complement of benthic indices for other embayments. Detailed information on recognizing California benthic habitats is provided in Chapter 5. Benthic species composition and abundances vary naturally from habitat to habitat and expectations for reference condition and measurements of deviation from reference should vary accordingly.

Sediment Samplers

A wide variety of sampling devices, such as grabs, cores, and dredges, have been used for collecting sediment. The SQO program requires the use of a grab sampler for sediment collection. The specific type of grab sampler used is often determined by the requirements of the monitoring program or habitat characteristics. Any grab sampler to be utilized should meet the following requirements: 1) it is constructed of material that does not introduce contaminants, 2) it creates a minimal bow wave while descending to the sediment surface to minimize disturbance of the flocculent layer, 3) it takes a sample with minimal disturbance to the sediment surface, 4) it does not leak sample or pore water during retrieval of sample, 5) the sample is easily accessed to verify sample quality and for removal of the sediment surface, and 6) samplers for benthic community condition meet size (surface area) requirements for the habitat being sampled.

The recommended grab sampler is the Van Veen or modified Van Veen grab (Figure 2.1). Grabs with a surface area of 0.05 m^2 are usually used in San Francisco Bay and 0.1 m^2 are used elsewhere. Depending on vessel configurations and study needs, smaller surface area grabs may be acceptable.

A Van Veen grab utilizes a lifting wire to close the jaws of the grab, while the sampler retains its depth of penetration, and can collect sediment up to a depth of 18 cm. Additionally, Van Veen grabs usually have sampler doors with a mesh covering, which minimizes disturbance of the flocculent layer on sediment surfaces. When the grab is open on descent, the mesh allows water to pass through the sampler, reducing the pressure wave created as the sampler descends to the sediment surface. Another significant advantage of a Van Veen sampler is its large access doors for visual inspection and removal of the upper undisturbed sediment layers. Equivalent grabs with smaller sampling surface area are suitable provided that the sediment sample is equivalent in quality to the Van Veen grab.

Sediment Collection

Multiple grab samples are usually required at each station to provide enough sediment for the assessment framework. There is no required order of sample collection, but the benthic infauna sample is often collected first as this sample is the most difficult to obtain. The benthic sample is also collected first to minimize disturbance of infaunal community from multiple grabs and to allow extra time for sample screening and processing. The inability to obtain a suitable benthic infauna sample is frequently the reason for unsuccessfully sampling a location.

Grab Evaluation

Grab sampling might be impossible or very difficult at some sites due to sediment or ocean conditions. Sediment type (percent sand) tends to be a significant determinant of achieving proper penetration depth. Increasing sand content typically decreases penetration depth, such that obtaining a minimum 5-cm penetration depth can be a challenge. Some sediment types (e.g., cobble, gravel, coarse sands) and localities (e.g., canyons, slopes, and rocky areas) could be difficult to sample. Sediments containing rocks and large/intermediate shell debris often prevent complete closure of the grab such that sediment washes out during retrieval.

Each grab sample must be inspected upon retrieval and determined to meet acceptability criteria before it can be used to provide sediment for analyses. The acceptability of a sample must be determined by inspection of the grab contents (Figure 2.2). An acceptable sample condition is characterized by a relatively even surface with minimal disturbance and little or no leakage of the overlying water. Heavily canted samples are unacceptable. Samples with a large amount of “humping” along the midline of the grab, which indicates washing of the sample during retrieval, are also unacceptable. However, some humping will be evident in samples taken from firmer sediment due to the closing action of the grab, such that humping is not necessarily evidence of unacceptable washing.

A

B

C

Figure 2.1 Sediment grab sampling equipment. A: Open Van Veen grab being deployed; B: Closed grab retrieval; C: Removing sediment with metal scoops.

Sediment penetration depth must be a minimum of 5 cm and should not exceed the capacity of the grab. In habitats where sediments are unusually soft (e.g., some estuary muds), it may be necessary to reduce weight to prevent over-topping the grab due to excessive penetration. All the grabs taken at a single station should be of similar sediment type. Marked variations in sediment type or grab penetration should be noted and brought to the attention of the field supervisor for a final determination of grab acceptability.

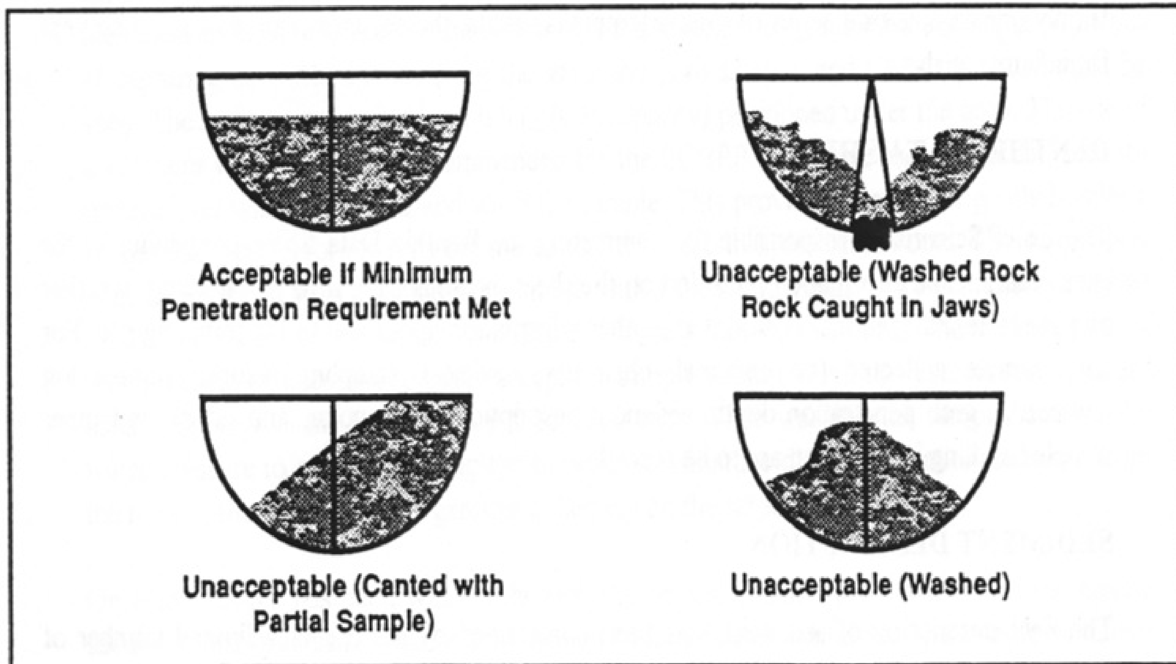


Figure 2.2. Examples of acceptable and unacceptable grab sample conditions (from Tetra Tech, Inc. 1986).

If sample condition is acceptable, the overlying water is drained off (and screened, for benthic community grabs) and the depth of penetration is determined and recorded. Precautions should be taken when draining the overlying water to minimize the loss of surface sediments. It is recommended that a siphon be employed for grabs used for toxicity and chemistry analysis; alternatively, the water may be drained off very slowly by slightly opening the jaws of the grab. The overlying water from grabs intended for infaunal analysis should be captured and screened along with the sediment from the grab, as this water may contain benthic macrofauna that are part of the sample.

Station Occupation and Grab Event Data

Station Occupation Data

Data on the station location and on conditions are recorded for every station. Collection of these data can be best accomplished by using electronic data capture with preformatted station sheets and as much computerized input as possible. This will simplify data recording, promote

consistency, minimize errors, and allow for rapid data collection. Computerized input could include automatic recording of GPS coordinates, anemometer readings, and fathometer readings. Manual recording on data sheets is acceptable if a computer system is unavailable or malfunctioning.

Station occupation data usually include:

- Station identification
- Date
- Time of arrival
- Collecting agency identification (or code)
- Vessel name
- System used for navigation
- Weather and sea conditions
- Salinity and temperature
- Notable observations or activities at or near the sampling site (e.g., surface sheens, vessel activity, boat cleaning, wildlife, etc.)
- Station fail code identifying reason for abandonment (if site is abandoned)

Some studies also measure bottom DO as an indicator of potential hypoxic conditions that could impact benthic communities.

Grab Event Data

All field measurements of sediment characteristics should be made before the sediment is removed from the grab for processing. Information about the grab sample and unusual incidents during sample collection should be documented. Examples of field descriptions and characterization of the sediments include coarse sand, fine sand, silt or clay, gravel, or a mixed grain size or color. The presence of non-aqueous-phase liquids, such as petroleum tar, and high percentages of shell hash should also be recorded, as should odors, such as hydrogen sulfide (the odor of rotten eggs), petroleum, humic and others, or a lack of noticeable odors. General sediment colors (i.e., black, green, brown, red, olive, or gray) or the presence of a surface sheen should also be recorded. Be aware that sunglasses can interfere with color determinations.

Onboard physical and chemical measurements of sediment parameters may be included, depending on the study design. These are best done before onboard sample processing and immediately, when possible, if there is known instability in the parameter to be measured. Examples of parameters include sediment pH, redox potential, and interstitial or pore water salinity. Sediment that has been disturbed by the measurement activities should not be included in samples for toxicity or chemistry analysis.

Grab event data usually include:

- Time of event (grab on bottom)
- Latitude and Longitude at time of event (grab on bottom)
- Depth of water (where grab on bottom)
- Depth of penetration of grab in sediment (to nearest 0.5 cm)
- Sediment composition (e.g., coarse sand, fine sand, silt or clay, gravel, or mixed grain size)
- Sediment odor
- Sediment color
- Presence of shell hash
- Sample types (e.g., sediment chemistry, sediment toxicity, or benthic community) taken from the grab

Quality Control Samples

The collection of quality control (QC) samples is recommended, as they facilitate an assessment of the accuracy, precision, representativeness, and bias of the study results. These samples also assess variability in contamination or toxicity associated with sampling procedures. QC samples cost the same to analyze as regular samples, so they should be used judiciously to address important components of the study. Examples of quality control samples are:

- **Field Blank:** Field blanks describe a group of QC samples used to measure sample contamination resulting from field procedures. For example, a travel blank (a type of field blank) consists of a sub-sample of clean sediment (provided by the analytical laboratory) that is transferred from its original container to another clean container during the field sampling procedure. This sediment can be analyzed for all analytes of interest concurrently with the field-collected sediments. An equipment blank (another type of field blank) consists of a clean sample or solvent that is exposed to the sampling device, sample containers, and scoops and then returned to the laboratory for analysis.

- **Field Replicate:** Replicates usually require a separate grab drop at a station. As the name implies these are additional samples taken at the collection site after collecting the original sample. They serve to assess heterogeneity within the station and uniformity in sample handling. Sediment samples are analyzed for the same constituents as the original. Common strategies utilize 5 to 10% replication among study stations. When available, replicate samples provide the ability to conduct statistical analysis between replicates providing a more accurate range of analyte concentrations at the collection site.

- **Duplicate:** Duplicates are split samples of sediment obtained from a single grab or sediment composite sample. Each sample is analyzed separately in order to assess variability associated with the sampling methods. Analytical labs also analyze duplicate

samples of sediment from the sample container. The lab duplicate samples are used to assess variability associated with homogenization and analysis of the sample.

Sample Processing

Cleaning Equipment

There are multiple potential sources of contamination during sampling, including boat surfaces, vessel exhaust, pelagic species introduction to benthic infauna, sediment carryover, or from skin and clothing of personnel. During sample collection, it is important that any contamination from these sources be minimized. This requires cleaning of all materials in contact with the samples (i.e., grab samplers, mixing bowls, utensils, and storage containers) and screening of intake water that is used for sample washing.

Proper handling and rinsing of samplers, utensils and mixing bowls are some of the simplest activities that minimize contamination. It is good practice to decontaminate all equipment between sites by washing, rinsing in ambient water, and then rinsing a final time with de-ionized (DI) water (and solvent, if allowed and necessary). Cover and/or store decontaminated gear such as samplers, mixing bowls, and utensils in a clean location between sites. Discard gloves used at the previous site. Put new gloves on upon arrival at a new site.

For multiple grabs within a site, rinsing with ambient water should suffice. The goal is to minimize contamination between grabs, so grab residuals should be discharged in a manner such that they drift away from the sampling spot. Use best judgment to ensure that grab residuals do not contaminate another nearby sampling station. Consult local authorities regarding discharge regulations within harbors and estuaries.

A method proven effective in cleaning equipment between sampling events includes the following steps:

1. Rinse equipment to remove all visible sediment.
2. Scrub all sampling utensils and mixing bowls with a detergent solution, either in a bucket or by using a spray bottle. Also wash all parts of the grab sampler with the detergent solution. Use care, because, depending upon the study and analysis (e.g., endocrine disruption, historical tracers), detergent residue may contaminate the sample and render the results invalid.
3. Completely rinse the grab, buckets, sampling utensils and bowls with raw water making sure they are clear of sediment and detergent residue.
4. Rinse items with 10% HCl followed by a rinse with pesticide-grade methanol. Note: Many vessel captains discourage the use of acid (because it corrodes metal bolts) and solvents (because they dissolve epoxy resins in fiberglass). A containment system should capture all residues (acid/solvent), and a hazardous material container should be used to store the used residue. See Coast Guard regulations regarding handling and storage of hazardous materials onboard a vessel.

5. Completely rinse the grab, buckets, sampling utensils, and bowls with DI water. Note that if insufficient DI water is available, a final rinse with ambient water is acceptable.
6. Cover all cleaned items (except for the grab) with aluminum foil or plastic until the next use to minimize exposure to airborne-particle contaminants. Choice of cover material depends upon the study and analytes being measured (i.e., it should not introduce contaminants that could affect the analyses). Rinse all cleaned items with ambient water before use.

Processing Benthic Community Samples

It is recommended that the entire contents of at least one grab at each station be dedicated to analyzing benthic community condition. Complete the grab event data form, wash the sediment sample from the grab, and then screen, relax, and fix the animals. Recommendations for these procedures are provided below. Note that the water used to wash samples should be filtered to prevent the accidental introduction of surface-water organisms. Be aware that different-sized grabs yield different infauna results.

Screening the Sediment

Typically, the grab contents are washed into a tub (≥ 70 L capacity) positioned under the grab, and the sediment is transferred from the tub into a screening box. Use of a sediment-washing table is recommended. The table provides a flat, smooth surface over which to spread and wash the sample, thus facilitating gentle break up of sediment clumps before they fall off the end of the table into the screening box. The screening box must be equipped with a stainless steel mesh with 1.0-mm openings (or 0.5-mm, for the San Francisco Bay). Wire diameter should be similar to that found in the U.S. standard 1.0-mm sieve (i.e., 0.0394 inches). The surface area of the screen should be adequate to easily accept the sample without clogging. Typical screen surface areas are 1500 to 2100 cm^2.

A smaller screen size or additional screens sizes are used in some studies (e.g., in the San Francisco Bay area, a 0.5-mm screen is utilized to separate smaller organisms from the sediment). These smaller screens are sometimes stacked below the 1.0-mm screen so that the material passes through each screen in sequence and the smaller organisms are captured and separated from the sediment. When all material smaller than 1.0 mm has passed through the top screen, the process is repeated with the finer screen until all material smaller than 0.5 mm has passed through. If the bottom screen (0.5 mm) begins to clog with sediment, the field crew ceases adding sample and gently runs the hose nozzle with low flow along the outside bottom of the 0.5-mm screen being careful not to lose sample by allowing water to escape over the top of the sieve. Note that if the original sample contains many shell fragments and/or worm tubes, the sediment sample should be added to the top screen (1.0 mm) in stages so that the screen does not become too full.

Water pressure must be controlled during washing to avoid damaging the organisms. Direct application of water from a hose without a nozzle to the material and organisms collecting on the screen should be avoided. A fan spray nozzle with a shut-off valve capable of adjusting pressure is typically used.

Note that the necessity of sampling from small craft may prohibit the ability to wash and screen onboard. In these cases, the samples may be screened and processed on land or from the side of the vessel at a temporary screening station established near the sampling location. To assure that the sample does not deteriorate, such off-site screening must be completed as soon as possible and no longer than 90 minutes after sample collection.

Transferring Samples to Containers

Once the sample has been washed through the screen, all the material (debris, coarse sediment, and organisms) retained on the screen is transferred into a sample container. When transferring the material to containers, great care should be taken to avoid damage to the organisms. The sample container should be filled to 50 to 70% of capacity with screened material. After the bulk of material has been transferred to the container, the screen should be closely examined for any organisms caught in the mesh. These should be carefully removed from the mesh, using pointed forceps or tweezers to avoid severing parts, and transferred to the sample container. Between samples, screens should be rinsed with water and scrubbed clean with a stiff-bristle brush. It is important to remove any accumulated debris from screens between samples to minimize the risk of cross-contamination.

A sample may be split between two or more containers if it is too large for one. Label the sample container with an external label containing the sampling agency name, station name, sample type, date, and container number (i.e., 1 of 1, 2 of 3, etc.). An internal label bearing the same information should be placed inside each infauna sample container. This label should be written in pencil or indelible ink on 100% rag paper, poly-paper, or other paper of a quality suitable for wet labels. The sample container must have a screw-cap closure and be sufficiently large to accommodate the sample material with a headspace of at least 30% of the container volume. To facilitate this, field crews should have a wide range of sample container sizes available to them.

Relaxing and Fixing the Specimens

All infaunal samples should be treated with a relaxant solution for approximately 30 minutes prior to fixation in 10% buffered formalin. Either an Epsom salts ($MgSO_4$) solution or a propylene phenoxytol solution (Table 2.1) may be used as a relaxant. Relaxant solutions may be used as the diluent water for the fixative or may be decanted off after relaxation is complete and replaced with diluted fixative. If it is used as diluent water, fill the sample container to 85 to 90% of its volume, close the container and gently invert it several times to distribute the solution. Leave the sample in the relaxant for 30 minutes. After 30 minutes, top off the container with enough sodium borate buffered formaldehyde to achieve a 10% formalin solution. Close the container, and gently invert it several times to assure mixing. Store the sample for return to the laboratory.

If the relaxant solution is not used as the diluent water, the relaxant must be removed from the sample container (e.g., by decantation) and replaced with 10% buffered formalin. After the 30 minutes of relaxation, decant the relaxant from the sample through a screen of the mesh size used previously to screen the sample. Remove all organisms from the screen and transfer them to the sample container. Fill the container with sodium-borate-buffered 10% formalin (rather than the undiluted buffered formaldehyde). Close the container, gently invert it several times, and store it for return to the laboratory. Samples should be kept in fixative for no less than 72 hours.

However, within two weeks of collection, they should be returned to the laboratory, washed, and transferred to a 70% ethanol preservative (see Chapter 4 for details). Thereafter samples can be held out of sunlight at relatively constant temperatures (< 30°C) for up to one year before the preservative must be refreshed or replaced.

Table 2.1. Relaxant and fixative stock solution recipes.

Solution	Composition
Epsom salts relaxant solution	1.5 kg Epsom salts ($MgSO_4 \cdot 7H_2O$) per 20 L of freshwater
Propylene phenoxytol solution	30 ml propylene phenoxytol to 20 L of seawater
Buffered formalin solution	50 g sodium borate ($Na_2B_4O_7$) per liter of formalin
Buffered 10% formalin solution	1 part buffered formalin to 9 parts fresh or saltwater

Processing Surface Sediment Samples for Chemistry and Toxicity Analysis

All the toxicity and chemistry analyses described in this manual are conducted on the surface sediment collected from the upper 5 cm of the grab/core sample. In contrast to the benthic infauna sample, the chemistry and toxicity samples are usually obtained from the same grab samples to maximize the comparability of the data. Up to 4 liters of sediment may be required for all the analyses, with the bulk of the sediment used for toxicity testing. As a result, multiple sediment grabs are almost always required to obtain sufficient sediment for analysis.

Except for samples for sediment-water interface toxicity tests (see following), the surface sediment is removed from the sampler using a non-contaminating scoop that is usually specially fabricated to remove only the desired depth of sediment. One typical scoop design resembles a metal box with the top and one end removed. The sides of the box are 5 cm high to provide a depth reference, a metal handle is attached, and the entire assembly is coated with a non-contaminating material (Figure 2.1).

Two strategies are typically used for sample processing and allocation of sub-samples: distributed and composited. In the distributed strategy, separate samples for chemistry and toxicity are obtained from the same (or subsequent) grabs and they are placed in containers specific for the analysis type. An effort is usually made to obtain comparable and representative samples for each type of analysis by obtaining sediment from multiple locations within each grab and using sediment from multiple grabs for each type of analysis. This subsampling strategy minimizes the potential for chemical contamination of the samples and provides the greatest flexibility in terms of materials used for sediment scoops and sample containers.

The composited sample processing strategy differs from the distributed strategy in that the sediment is placed into an intermediate mixing container and homogenized prior to filling the storage jars. From a toxicological perspective, it is preferable that the sediment be composited and homogenized prior to distribution into the sample containers. This method maximizes

comparability of the chemistry and toxicity samples and may provide more options for the location of the sample allocation step (e.g., homogenization and filling of sample jars can take place within a laboratory area of the ship, which may be a cleaner environment). Both the mixing container and utensil (e.g., spoon, spatula) used for taking the sub-sample should be of non-contaminating material. An inert coating, such as Teflon, would be acceptable for the mixing bowl and utensils.

Unless the grab sampler is coated with a non-contaminating material, sediment in contact with, or within 1 cm of, the metal sides should be avoided to prevent sample contamination. Furthermore, to prevent contamination during collection of sub-samples, all containers, scoops, and related gear should be covered when not in use.

Processing Sediment Samples for Sediment-Water Interface Toxicity Tests

The Sediment-Water Interface (SWI) test is used to assess toxicity of solid phase sediment samples using the embryo or larval stages of marine and estuarine invertebrates. This test is designed to be conducted on a relatively undisturbed core sample containing the upper 5 cm of sediment, which requires the use of the special sample processing methods described below. If the study design calls for the SWI test to be conducted on homogenized samples, then the sample processing methods described in the previous subsection should be employed.

Intact sediment can be taken from the grab sampler using a polycarbonate core (approximately 7.5 cm inside diameter). This subsample must be the first sediment taken from an undisturbed grab. The core is pressed 5 cm into the sediment and a pre-cleaned acrylic plate, or a gloved hand is inserted under the bottom of the core to prevent loss of sample as the core is removed. It is convenient to mark the height (5 cm) for reference around the outside of the core. After the core is removed from the grab, it is gently wiped of exterior sediment and the bottom is capped quickly with a polyethylene plastic cap; the top is then capped.

Core subsample integrity is verified by the presence of sediment overlying water and the required depth of sediment. If an inordinate volume of sediment is lost, the sample is discarded and a new one collected. A small hole in the top cap relieves positive pressure on the sample and minimizes leakage as the cap is attached. Once capped, the outside of the core is washed, and the core is placed upright in a cooler for storage and transport. Care must be taken to minimize tilting, shaking or vibrating these cores during transport. Precautions should also be taken to prevent contamination of the core contents by water from melting ice during storage.

Sample Storage

Recommended conditions for sample handling and storage are listed in Table 2.2. Additional detailed analysis-specific recommendations are presented below the table.

Table 2.2. Recommended sample sizes, containers, preservation, and storage for sediment.

Sample Type	Volume (ml)[a]	Container[b]	Preservation Technique		Storage (months)[c]
			Transport	Storage	
Grain Size	70	HDPE or Glass	Wet ice then 4ºC	4ºC	6
Total Organic Carbon	135	HDPE or Glass	Wet ice then 4ºC	-20ºC	6
Metals	70	HDPE or Glass	Wet ice then 4ºC	-20ºC	12
Total Mercury	35	Glass	Wet ice then 4ºC	-20ºC	6
Organics	135	Glass	Wet ice then 4ºC	-20ºC	12
Chemistry Archive	200	Glass	Wet ice then 4ºC	-20ºC	
Toxicity		HDPE or Glass	Wet ice then 4ºC	4ºC	1
Amphipod Survival	1500				
Mussel Embryo Development	1500				
Polychaete Growth	1000				

[a] Minimum volume to conduct analyses or a single toxicity test with appropriate controls.
[b] Recommended container, but other types are suitable.
[c] Recommended storage time used by multiple programs.

- **Sediment Grain Size:** This sample should be placed in a glass or high-density polyethylene (HDPE) container or polyethylene bag, taking care to leave an air space at the top. Samples should be stored at > 0 to 4°C by placing them on wet ice or in a refrigerator until submitted to the laboratory. **Do not freeze these samples.**

- **Total Organic Carbon:** This sample should be placed in a glass or HDPE container with a Teflon-lined lid. The container should be 75 to 80% full, taking care to leave an air space at the top to prevent breakage of the container due to expansion of the sample during freezing. Samples should be stored at > 0 to 4°C by placing them on wet ice or in a refrigerator and must be frozen within 24 hours.

- **Metals:** This sample should be placed in an acid-cleaned glass or HDPE container with a Teflon-lined lid. This container should be 75 to 80% full, leaving an air space at the top to prevent breakage of the container due to expansion of the sample during freezing. Samples should be stored at > 0 to 4°C by placing them on wet ice or in a refrigerator and must be frozen within 24 hours.

- **Organics:** This sample should be placed in a solvent-rinsed (or pre-certified clean) glass container with a Teflon-lined lid. The container should be 75 to 80% full, taking care to leave an air space at the top to prevent breakage of the container due to expansion of the sample during freezing. Samples should be stored at > 0 to 4°C by placing them on wet ice or in a refrigerator and must be frozen within 24 hours.

- **Chemistry Archive:** This sample should be placed in a solvent-rinsed (or pre-certified clean) glass container, with a Teflon-lined lid. The container should be 75 to 80% full, taking care to leave an air space at the top to prevent breakage of the container due to expansion of the sample during freezing. Samples should be stored at > 0 to 4°C by

placing them on wet ice or in a refrigerator and must be frozen within 24 hours. Caution is advised when using Teflon lid liners for samples intended for potential analysis of perfluoroalkyl compounds (PFAS), which are related to Teflon.

- **Toxicity:** This sample should be stored in a glass or a HDPE plastic container, such as a wide mouth jar or heavy-duty polyethylene bag, taking care to leave an air space at the top. Samples should be stored in the dark at > 0 to 4°C by placing them on wet ice or in a refrigerator until returned to the laboratory. **Do not freeze these samples.** Samples should be analyzed within two weeks of sampling, if possible, with a maximum storage time of 4 weeks.

- **Toxicity - *Sediment-Water Interface Test*:** This sample should be maintained intact in its core and sealed at the bottom to prevent leakage. The core should remain upright, to not disturb stratification and be maintained in the dark at > 0 to 4°C. If the cores are cooled by ice, precautions should be taken to prevent contamination of the cores by melting ice. **Do not freeze these samples.** Samples should be analyzed within two weeks of sampling, if possible, with a maximum storage time of 4 weeks.

Chapter 3: Sediment Chemistry

Sediment chemistry is an essential line of evidence (LOE) required for sediment quality assessment. The Chemistry LOE, which is the California Sediment Quality Objectives (CASQO) chemistry endpoint, helps determine the type of chemical exposure and its potential for producing adverse biological effects. Determination of the Chemistry LOE is comprised of two main components: 1) measurement of a suite of constituents and 2) interpretation of the results using indices of chemical exposure that are based on sediment quality guidelines (SQGs).

This chapter provides computational tools for determining the Chemistry LOE category. The data analysis procedure described includes calculation of chemical contamination indices based on two types of SQGs: 1) the California Logistic Regression Model (CA LRM) and 2) the Chemical Score Index (CSI). Integration of these two indices yields the Chemistry LOE. At the end of the chapter, an example of the step-by-step process for determining the Chemistry LOE category is provided.

Objectives

The objective of this chapter is to describe the sediment-chemistry analyses needed to apply the CASQO framework. The information in this chapter is intended to supplement laboratory protocols commonly used for monitoring California's subtidal marine and estuarine habitats by indicating those constituents and methods needed to obtain data consistent with the requirements of the CASQO framework.

Scope

The methods described in this chapter focus only on the sediment constituents that must be assessed to conduct the CASQO station assessment. The lack of description of specific contaminants or methods is not intended to imply that they are not important as other elements of a sediment quality assessment program. As with any program, the specific study design and project objectives should determine what is measured.

Sediment Chemistry Constituents

To generate the Chemistry LOE, a specific set of sediment chemistry constituents should be measured. These are provided in Table 3.1. The recommended maximum reporting limits (RLs) listed for each constituent are based on the CSI classification ranges and do not necessarily reflect the maximum performance achievable with available analytical methods. The concentrations associated with each RL are expressed on a dry-weight basis.

Table 3.1 should not be interpreted as an exhaustive list of all analytes that might be of interest in a sediment quality assessment study. Each program will need to determine what other analytes (e.g., certain pesticides) might be of value, depending on the objectives of the monitoring program. In addition, it should be noted that some other analytes that are not required by the Chemistry LOE should also be measured to help interpret the Toxicity and Benthic Community LOEs. These include total organic carbon (TOC) and percent fines.

Table 3.1. Constituents to be analyzed for sediment chemistry determination within the CASQO framework and their corresponding recommended maximum reporting limits (RLs).

Target Analyte	Maximum RL
Metals	
Cadmium (mg/kg)	0.09
Copper (mg/kg)	52.8
Lead (mg/kg)	25.0
Mercury (mg/kg)	0.09
Zinc (mg/kg)	60.0
Polycyclic Aromatic Hydrocarbons (PAHs)	
Low Molecular Weight PAHs:	
Acenaphthene (μg/kg)	20.0
Anthracene (μg/kg)	20.0
Phenanthrene (μg/kg)	20.0
Biphenyl (μg/kg)	20.0
Naphthalene (μg/kg)	20.0
2,6-dimethylnaphthalene (μg/kg)	20.0
Fluorene (μg/kg)	20.0
1-methylnaphthalene (μg/kg)	20.0
2-methylnaphthalene (μg/kg)	20.0
1-methylphenanthrene (μg/kg)	20.0
High Molecular Weight PAHs	
Benzo(a)anthracene (μg/kg)	80.0
Benzo(a)pyrene (μg/kg)	80.0
Benzo(e)pyrene (μg/kg)	80.0
Chrysene (μg/kg)	80.0
Dibenz(a,h)anthracene (μg/kg)	80.0
Fluoranthene (μg/kg)	80.0
Perylene (μg/kg)	80.0
Pyrene (μg/kg)	80.0

Table 3.1. (continued).

Target Analyte	Maximum RL
Organochlorine Pesticides	
Alpha Chlordane (μg/kg)	0.50
Gamma Chlordane (μg/kg)	0.54
Trans Nonachlor (μg/kg)	4.6
Dieldrin (μg/kg)	2.5
o,p'-DDE (μg/kg)	0.50
p,p'-DDE (μg/kg)	0.50
o,p'-DDD (μg/kg)	0.50
p,p'-DDD (μg/kg)	0.50
o,p'-DDT (μg/kg)	0.50
p,p'-DDT (μg/kg)	0.50
Polychlorinated Biphenyls (congener number)	
2,4'-Dichlorobiphenyl (μg/kg) (8)	3.0
2,2',5-Trichlorobiphenyl (μg/kg) (18)	3.0
2,4,4'-Trichlorobiphenyl (μg/kg) (28)	3.0
2,2',3,5'-Tetrachlorobiphenyl (μg/kg) (44)	3.0
2,2',5,5'-Tetrachlorobiphenyl (μg/kg) (52)	3.0
2,3',4,4'-Tetrachlorobiphenyl (μg/kg) (66)	3.0
2,2',4,5,5'-Pentachlorobiphenyl (μg/kg) (101)	3.0
2,3,3',4,4'-Pentachlorobiphenyl (μg/kg) (105)	3.0
2,3,3',4',6-Pentachlorobiphenyl (μg/kg) (110)	3.0
2,3',4,4',5-Pentachlorobiphenyl (μg/kg) (118)	3.0
2,2',3,3',4,4'-Hexachlorobiphenyl (μg/kg) (128)	3.0
2,2',3,4,4',5'-Hexachlorobiphenyl (μg/kg) (138)	3.0
2,2',4,4',5,5'-Hexachlorobiphenyl (μg/kg) (153)	3.0
2,2',3,4,4',5,5'-Heptachlorobiphenyl (μg/kg) (180)	3.0
2,2',3,4',5,5',6-Heptachlorobiphenyl (μg/kg) (187)	3.0
2,2',3,3',4,4',5,6-Octachlorobiphenyl (μg/kg) (195)	3.0

Sediment Chemistry Methodology

Recommendations for sample preparation, extraction/clean-up, and analysis for each of the CASQO sediment chemistry constituents are provided in Table 3.2.

The use of USEPA-approved methods such as the "*Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*" (also known as SW-846; (USEPA 2008a)) is recommended. If standard methods are not available, approval of alternative methods should first be obtained from the State Water Resources Control Board. Additional methods may be acceptable if they produce results that are at or below the desired reporting limits and are comparable to results that would be generated by EPA SW-846.

Table 3.2. Commonly used and recommended (USEPA SW-846[1]) extraction, clean-up, and determinative methods for sediment chemistry analysis.

Analyte	Extraction/Digestion	Clean-up	Determinative
Total Organic Carbon			Carbonaceous Analyzer EPA 9060, 5310
Metals			
Cd, Cu, Pb, Zn	Nitric/Hydrochloric Acid		Flame Atomic Absorption (FLAA) EPA 7000 Graphite Furnace Atomic Absorption (GFAA) EPA 7010 Inductively Coupled Argon Plasma-Optical Emission Spectrometry (ICP-OES) EPA 6010 Inductively Coupled Argon Plasma-Mass Spectrometry (ICP-OES) EPA 6020
Hg	*Aqua Regia* Digestion EPA 7471 Acid Digestion EPA 7474		Cold Vapor Atomic Absorption (CVAA) EPA 7471 Cold Vapor Atomic Fluorescence (CVAA) EPA 7474
Organics	Soxhlet Extraction EPA 3540 Sonication Extraction EPA 3550 Microwave Assisted Extraction EPA 3051 Pressurized Fluid Extraction EPA 3545	Sulfur Removal EPA 3660 Gel Permeation Chromatography EPA 3640 Alumina EPA 3600, 3610 Florisil EPA 3620	
PAHs			GC-MS (in SIM mode) EPA 8121 Flame Ionization Detection EPA 8015
Pesticides			GC-MS EPA 8270 GC-ECD EPA 8081
PCBs		Strong Acid EPA 3565	GC-MS EPA 8270; 1668C GC-ECD EPA 8082

[1]Method reference refers to the latest promulgated revision of the method, even though the method number does not include the appropriate letter suffix.

Sample Handling, Preservation, and Storage

Since most analyses require trace-level detection limits (i.e., parts per billion; ppb), mitigation of contamination sources is paramount. Because of the challenges associated with trace-level measurements, it is recommended that the laboratory conducting these analyses have experience in quantifying these constituents at comparable RLs (see Table 3.1) on a routine basis.

Caution should be taken to avoid contamination at each stage of sample collection, handling, storage, preparation, and analysis. All sample containers should be purchased pre-cleaned or be pre-washed in accordance with methods described below or comparable methods. Sample containers and labware should be cleaned and stored according to sample type and analyte of concern. Handling or touching of insides of glassware should be avoided, as gloves or utensils can introduce residues such as plasticizers to the samples.

Proper rinsing of containers is necessary to eliminate soap residues that can interfere with analysis of certain analytes. It is recommended that Teflon-coated squirt bottles be employed to hold solvents or acids used for rinsing sample containers and labware. Labware that does not appear clean or that is etched should be removed from trace analysis work. Except for volumetric labware, high temperature glass labware for trace organics analysis work should be baked at 500°C to remove contaminant residues. Caution is advised when using Teflon-coated squirt bottles to rinse containers and labware to be used for analysis of perfluoroalkyl compounds (PFAS), which are related to Teflon.

When samples are received by the laboratory, sample acceptance requirements specified in the project Quality Assurance Project Plan (QAPP) should be adhered to in order to ensure sample integrity. Chain of Custody (COC) procedures should be conducted by personnel who are properly trained and authorized to handle incoming sample bottles and records. The following should be verified:

- Sample identification (i.e., these should be congruent between the sample container and the field sheet)
- Acceptable condition of sample bottles (i.e., none should be broken or improperly capped)
- Sample receipt within holding time (refer to Table 2.2)
- Appropriate sample preservation and storage to ensure stability of the analyte
- Sample receipt temperature

When applicable, any safety hazards associated with the samples should also be noted and documented, and the appropriate personnel should be notified.

Sediment samples should be stored in the dark at 4ºC, on ice, or frozen at -20ºC, as required for the analytes prior to extraction. Due to the unstable nature of some of the analytes of interest, it is suggested that holding times be as short as possible and that extracts are analyzed as recommended in SW-846. In all cases, an analysis must start prior to expiration of the holding time.

Sample Preparation and Analysis

Multiple analyses are often conducted on different sediment aliquots. Therefore, it is important to ensure that sediment is well homogenized before aliquoting samples (e.g., for metals or for organics analyses). This includes re-incorporating any overlying water into the sample before taking an aliquot.

Recommended determinative methods for the CASQO target analytes are listed in Table 3.2. The analytical methods selected should be ones that are routinely conducted by the contracted laboratories. The laboratories should be familiar not only with the methods, but also with the guidelines and quality controls necessary for the analytes in question. Also, as a general recommendation, the analytical method chosen for a given analyte should be one that can achieve the target RL or lower.

Sediment Grain Size

Grain size is the measure of particle size distribution for sediments. This is usually reported as percent fines (silt + clay) and percent coarse grain (sand). However, distributions are also measured and reported in *phi* size categories, which can be translated into fines and coarse-grain groups.

The primary consideration in conducting grain-size analysis is the need to use a standardized methodology and standard sieve mesh sizes for the analysis. Grain size distribution should be measured by either laser- and light-scattering procedures, pipette or by hydrometer analysis (ASTM D7928-17). As multiple technologies for particle size analysis are in common use, the results may not be comparable between them. To ensure comparability of data, similar methods should be used for all analyses within a study and SOPs should be established to document specific procedures. Regardless of which procedure is chosen, gravel should first be separated from finer particles using a size 2000-µm mesh sieve (and then quantified), and the pass-through sediment should then be separated from finer particles using size 1000-µm mesh (and then quantified). For the former procedure, the material that passes through this second sieve is then subjected to laser/light-scattering assessment of the distribution of the remaining size particles. If the pipette method is chosen, a third screening of sediment should be done with size 63-µm mesh to isolate the fines. The pass-through material is then subjected to pipette analysis of size-particle distribution.

Analyte-Specific Recommendations

Total Organic Carbon Preparation and Analysis

To prepare samples for TOC analysis, frozen sediments are thawed to room temperature and homogenized before being dried in an oven at 60ºC overnight. The dried samples are exposed to concentrated hydrochloric acid vapors in a closed container to remove the inorganic carbon. TOC samples can be analyzed by various methods that include high temperature combustion and UV/persulfate oxidation. Analytical grade acetanilide (99.95+ %) is recommended as an external standard for TOC. A certified reference material, such as the PACS-1 marine sediment (National Research Council of Canada), is recommended for evaluating analytical performance.

Metals Sample Preparation

Samples for metals may be prepared for analysis either as wet sediment, dried at room temperature, oven dried, or freeze-dried. If room temperature or oven drying is used, care should be taken in the drying process to minimize volatilization of analytes in the samples by not exceeding a temperature of 60ºC. To avoid potential problems, analysis of wet or freeze-dried samples is preferred. Sediment metals results must be reported per dry weight. To do this, a separate aliquot from the original sample is taken and dried to determine the moisture content.

Sample Digestion for All Metals Except Mercury

The recommended digestion method for target metals other than mercury is the strong acid digestion (EPA Method 3050 (USEPA 2008a) or EPA 1638 Modified). The alternative procedure, total acid digestion, is not recommended because it uses hydrofluoric and perchloric acids and can result in safety hazards. It can also result in high dissolved solids that cause

physical and spectral interferences for all the determinative methods. Such interferences can be severe enough to require dilution of the digestate, resulting in higher reporting limits. Furthermore, the development of the CASQO chemical indices was based on analyses of metals data obtained using strong acid digestion. As such, use of a method other than strong acid digestion may affect the accuracy of the Chemistry LOE determination.

Sample Digestion and Analysis for Total Mercury

EPA Methods 7471 and 245.5 (USEPA 1991, 2008a) can be used for sediment digestion for mercury analysis. These methods use *aqua regia*, a mixture of one part concentrated nitric acid and three parts concentrated hydrochloric acid, as part of the digestion process and result in quantitative recoveries for total mercury in marine sediments. The use of *aqua regia* rather than the nitric/sulfuric acid mix specified in EPA Method 245.1 (USEPA 1991) is effective for highly organic sediment samples.

Metals Analysis

A variety of methods are available to quantify metals in the samples (Table 3.2). However, most monitoring programs use inductively coupled plasma mass spectrometry (ICPMS). Cold Vapor Atomic Absorption or Cold Vapor Atomic Fluorescence EPA 7471 (USEPA 2008a) are the recommended techniques for analysis of mercury in marine sediments.

Organics Preparation

Clean-up procedures are usually necessary before the analysis of organic compounds from extracts of marine sediments to maximize accuracy and precision of results. Methods for clean-up are detailed in Table 3.2 and include sulfur removal, chromatography, and the use of strong acid/oxidizers.

Sample preparation for organics analyses generally involves extraction from the sample matrix followed by isolation and concentration of target analytes prior to instrumental analysis. Common extraction procedures for organic contaminants in marine sediments are taken from USEPA SW-846 (Table 3.2), including: Soxhlet Extraction, Sonication Extraction, Microwave Assisted Extraction, and Pressurized Fluid Extraction (also called Accelerated Solvent Extraction (ASE); EPA 3545;(USEPA 2008a)). ASE has shown promise to maintain or improve on extraction efficiencies while greatly reducing solvent volumes and extraction times. It should be noted that modifications to any of the methods listed may be necessary to achieve low-level detection limits. Modifications can include reducing the final volumes cited by the method, starting with larger sample sizes, or both.

As with metals, organic results must be reported on a dry-weight basis. Therefore, a separate sub-sample or aliquot from the original homogenized sediment sample should be analyzed for moisture content.

Organics Analysis

Polychlorinated Biphenyl (PCB) congeners and organochlorine pesticides are usually analyzed by either dual-column gas chromatography electron capture detection (GC-ECD; EPA 8081) or gas chromatography mass spectrometry (GC-MS; EPA8270) in the selected ion monitoring

(SIM) mode (EPA 8082). Polycyclic Aromatic Hydrocarbons (PAHs) are generally analyzed by GC-MS or flame ionization detection (EPA 8015).

Quality Assurance/Quality Control

The following section provides recommendations on the Quality Assurance/Quality Control (QA/QC) elements that should be included in a sediment chemistry assessment program to generate high quality data. In addition to confirming data quality, these elements can also provide insight into problems with the data so that appropriate corrective actions can be taken.

From the standpoint of analytical methodology, it should be noted that a significant portion of what is recommended in this chapter is based on "*Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*," SW-846 (USEPA 2008a). While modifications of standard analytical procedures may also be acceptable for application to the CASQO framework, any such methods should be demonstrated to provide results with levels of precision and accuracy that equal, or exceed, those generated through the standard protocols. As such, when modified methods for sediment chemistry analysis are employed, the use of "performance-based methodology" is strongly encouraged in order to achieve a level of data quality consistent with the CASQO Program. This approach typically involves development of Data Quality Objectives (DQOs; see below) that are relevant for, and compatible with, the desired use in terms of timeliness, completeness, accuracy, and precision.

Analysis Sensitivity

Sediment chemistry analyses for application within the CASQO framework must be conducted with a degree of sensitivity sufficient to generate a meaningful Chemistry LOE. For each analyte, it is necessary to identify the minimum level at which there is high technical confidence in the quantified result (i.e., a threshold above which there is a low probability of either a false positive or false negative). This is accomplished by defining a RL for each analyte. The RL is the minimum concentration that can be measured by a given lab, using a given methodology, without risking substantial interferences. Any result above the RL can be reported in the project database without any sort of qualification stating that it is an *estimated* quantity.

The RL is related to another measure of sensitivity, the Method Detection Limit (MDL), which can be determined empirically based on the standard deviation of low-level matrix spike responses. The MDL indicates the level of "noise" inherent in the analytical methodology used by a given laboratory. Any results falling below the MDL cannot be distinguished from zero and therefore should be qualified as such, with no reporting of numerical values (see below). Knowledge of the MDL achievable by a given laboratory is crucial to understanding whether sufficiently low concentrations of the analyte can be reliably quantified for ultimately deriving the Chemistry LOE.

While RLs are often around 5 to 10 times higher than corresponding MDL for a given analyte, the establishment of RLs is ultimately at the discretion of the laboratory conducting the analyses.

Recommended Reporting and Detection Limits

For use of sediment chemistry results within the CASQO framework, it is recommended that the RLs for all the target sediment chemistry constituents be on par with those presented in Table

3.1. Laboratories should report the RL and the MDL for each analysis. This information should also be included in the project dataset. The MDL is important to include because it facilitates Chemistry LOE calculations in situations in which target analyte results fall below reporting limits (see below).

Result Qualifier Codes

Results reported at the RL or above correspond to lower uncertainty and thus are believed to be more accurate than those below the RL. However, detectable levels of target constituents between the RL and MDL can also provide some valuable information. Thus, it is recommended that data be reported as follows:

- if $x \geq RL$, report the determined concentration; no data qualification is necessary
- if $MDL \leq x < RL$, report the estimated concentration; also add a data-qualifier that indicates a lower level of confidence in the result, such as *data not quantifiable* (DNQ)
- if $x < MDL$, do not report a value; report *non-detect* (ND) in the qualifier section

Data Quality Objectives

It is very important to establish the validity of the sediment chemistry data prior to using them to generate the Chemistry LOE. A robust dataset should include a full suite of QA/QC samples that are indicative of how successfully sampling and laboratory analytical procedures were carried out. DQOs should also be set for each of the QA/QC sample types. Examples of acceptable DQOs that have been employed in marine sediment chemistry studies are provided in Table 3.3.

The output for the QA/QC samples in each analytical run should be compared to the pre-established DQOs as a measure of the reliability of that run's results. In addition to this, as a matter of course, all laboratories should keep detailed notes during sample preparation and analysis. All this information will be used to validate the data and troubleshoot any problems.

For each sample batch (traditionally defined as a group of up to 20 samples), it is recommended that at least one each of the following QA/QC sample types be included in the analytical run:

- A *blank* to determine the likelihood that samples in the batch have been contaminated
- A *matrix spike* to evaluate the potential for interference(s) between components of the sample matrix and the analysis of the target constituent
- A *duplicate* to estimate the precision of the results, by calculating the relative percent difference (RPD)
- A *standard* using certified reference material (CRM) to assess the accuracy of the analytical procedure

Table 3.3. Example of Data Quality Objectives (DQOs) for sediment chemistry analysis (adapted from the Bight '03 QAPP). RPD = Relative Percent Difference.

QA/QC Sample Type	Data Quality Objective
Blanks	
Frequency	1/batch
Accuracy	< MDL
Certified Reference Material (CRM)	
Frequency	1/batch
Accuracy	Within ±30% of certified value for 80% of analytes
Matrix Spikes	
Frequency	1/batch
Accuracy	Within ±30% of true value
Precision	RPD < 30%
Sample Duplicates	
Frequency	1/batch
Precision	RPD < 30%

Data Validation

Before using any analytical results in calculations to derive the Chemistry LOE, each data report should be carefully inspected to determine the validity of the analytical run and the completeness of data reporting. Recommended data validation measures include confirming the following:

- Reporting units and numbers of significant figures are correct
- MDLs and RLs have been reported by the laboratory for each analyte and are within the recommended limits provided in Table 3.1
- Initial and continuing instrument procedural blank levels are consistent with laboratory QA/QC guidelines
- Initial and continuing calibration of laboratory instrumentation meets laboratory QA/QC guidelines
- QA/QC samples (blanks, duplicates, matrix spikes, percent recovery surrogates, and CRM/Standard Reference Material (SRM)) have met or exceeded DQOs
- Reported concentrations for each analyte fall within "environmentally-realistic" ranges, as deduced from previous studies and expert judgment

Corrective Actions

If the data validation process reveals a problem, this information in combination with the review of laboratory comments can help to identify and rectify it. Data that are deemed suspect because of failure to meet DQOs should be re-evaluated and flagged with data qualifiers, where

appropriate. Depending on the severity of the problem, re-sampling and re-analysis of some or all samples may be necessary. Any corrective actions should be taken before subsequent sample batches are analyzed, and technical interpretation/reporting and use of the data should begin only after the full QA/QC review has been completed.

Data Management and Reporting

Once data quality has been deemed satisfactory, all raw data (including the result of all quality-assurance samples) should be entered in a project database whose format is standardized and therefore accessible to other parties. A report summarizing the process and outcome of data evaluation should also be prepared to accompany the database.

Sediment chemistry data should be stored in a database using a standard format that will be accessible to other users. Several possible formats are available, and some examples include the databases for the State Water Board's Surface Water Ambient Monitoring Program (SWAMP) and the Southern California Bight Regional Monitoring Surveys. A report summarizing the QA/QC review of the data package should be prepared and made available to potential users of the database. Laboratory data and accompanying explanatory narratives should also be archived.

Reports documenting the results of the QA/QC review of a data package should summarize all conclusions concerning data acceptability and note significant problems. These reports are useful in providing data users with a written record of any data concerns and a documented rationale for why certain qualified data were either accepted as estimates or rejected.

At a minimum, the following items should be addressed in a QA/QC report:

- Summary of overall data quality, including a description of data that were qualified.
- Brief descriptions of analytical methods and the method(s) used to determine MDLs.
- Description of data reporting, including any corrections made for transcription or other reporting errors, and description of data completeness relative to objectives (e.g., 90% complete) stated in the QAPP.
- Descriptions of initial and ongoing calibration results, blank contamination, and precision and bias relative to QAPP objectives and stated DQOs (including tabulated summary results for CRMs, laboratory control materials, and matrix spikes/matrix spike duplicates).

Calculation of the Chemistry LOE

The Chemistry LOE is based on a combination of two sediment chemistry indices that determine the magnitude of chemical exposure at a site. The chemistry indices are based upon two types of sediment quality guideline approaches: 1) a logistic regression model calibrated to California data (CA LRM; (Bay *et al.* 2012)) and 2) the Chemical Score Index (CSI; (Ritter *et al.* 2012)).

The CA LRM was developed using an EPA logistic regression modeling approach that estimates the probability of toxicity based on the chemical concentration (Field *et al.* 2002; USEPA 2005b). The CSI uses chemistry data to predict the occurrence and severity of benthic community disturbance. Index-specific response ranges are applied to each index to classify the

result into one of four chemical exposure categories: Minimal, Low, Moderate, and High. The resulting exposure categories are assigned a score of 1 to 4 (e.g., Minimal Exposure = 1) and the average of the scores for each chemistry index is used to determine the overall Chemistry LOE category.

The specific chemical constituents used in the indices were selected as part of the CASQO tool-development process and are listed in Table 3.4. Note that each index uses a subset of the constituents. Selection of these constituents was based on multiple factors including data availability and index performance. It should be noted that omission of other contaminants from the list in Table 3.4 does not imply that such contaminants are not potentially important factors influencing sediment quality.

Because the CA LRM and CSI indices are based on data for specific constituents, substitutions or omissions thereof may result in an inaccurate determination of the Chemistry LOE and are therefore not recommended. If for some reason it is necessary to omit one or more of these constituents, this information should be reported to the study manager and used to qualify the CASQO results accordingly.

Chemical Category Sums

Six of the constituents in Table 3.4 represent the sum of multiple chemicals (i.e., low molecular weight PAH (LPAH), high molecular weight PAH (HPAH), total PCBs, total DDTs, total DDEs, and total DDDs). The specific compounds comprising the sums of the PAH and PCB groups are listed in Table 3.1. Total DDTs represents the sum of p,p'-DDT and o,p'-DDT; total DDEs represents the sum of p,p'-DDE and o,p'-DDE; and total DDDs represents the sum of p,p'-DDD and o,p'-DDD. The compounds making up each group were based on those used in the National Oceanic and Atmospheric Administration (NOAA) Status and Trends program.

Table 3.4. CASQO sediment chemistry target constituents, the Chemistry LOE indices for which they are used, and example values used for the demonstration calculations in this chapter.

Sediment Constituent	Applicable Index		Example Concentration
	CSI	CA LRM	
Cadmium (mg/kg)		X	0.15
Copper (mg/kg)	X	X	43.6
Lead (mg/kg)	X	X	33.5
Mercury (mg/kg)	X	X	1.37
Zinc (mg/kg)	X	X	45.4
HPAH (µg/kg)	X	X	1672
LPAH (µg/kg)	X	X	261
Alpha Chlordane (µg/kg)	X	X	3.1
Gamma Chlordane (µg/kg)	X		2.4
Dieldrin (µg/kg)		X	1.7
Trans Nonachlor (µg/kg)		X	2.5
DDDs, total (µg/kg)	X		6.7
DDEs, total (µg/kg)	X		2.7
DDTs, total (µg/kg)	X		10.6
4,4'-DDT (µg/kg)		X	2.5
PCBs, total (µg/kg)	X	X	22.7

The sums for HPAH, LPAH, DDTs, DDDs, and DDEs are calculated by adding the reported (i.e., quantified) value of each individual compound, expressed on a dry weight basis. Compounds qualified as non-detected are treated as having a concentration of zero for the purpose of summing. If all components of a sum are non-detected, then the highest reporting limit of any one compound in the group should be used to represent the sum value.

A slightly different summation method is used for the PCBs to compensate for the use of a shorter list of PCB congeners than the NOAA program. The concentrations for the individual PCB congeners are summed as described above. This total PCB sum is then multiplied by a correction factor of 1.72 to approximate the value obtained using the larger NOAA list. A reduced list of congeners was selected for the CASQO program to provide greater compatibility with California historical data sets, which often have a reduced congener list.

Example of Chemistry LOE Calculation

This section demonstrates the process for data preparation and calculation to generate the Chemistry LOE for the CASQO assessment framework. The data used in this demonstration are shown in Table 3.4. They represent all the sediment chemistry constituents that are recommended for inclusion within the CASQO framework. The sample data provided are within ranges that are typical for each constituent for the sediment of California marine and estuarine habitats.

All the necessary calculations can be carried out using a standard desk calculator or a spreadsheet program, such as Microsoft Excel. For convenience, the Southern California Coastal Water Research Project (SCCWRP) website provides a spreadsheet tool for these calculations.

Note that this spreadsheet tool is periodically updated to incorporate input from users; the current version can be found in the *Sediment Quality Assessment Tools* section of the *Sediment Quality* research area page at www.sccwrp.org.

Data Preparation

The first step in the Chemistry LOE calculations is to confirm that the data are in the proper format. All constituents must be expressed on a sediment dry-weight basis. Specifically, all metals should be in mg/dry kg and all organic constituents should be in µg/dry kg. Note that if calculations using non-detected (ND) analytes are necessary, an estimated value must be used. One estimation approach is to use 50% of the MDL for any samples with ND results for that analyte; however, the previous section should be consulted for addressing ND values within summed groups of constituents.

Calculation of Component Indices

To generate the Chemistry LOE score, the values of the CA LRM and the CSI must first be calculated. Those values are then integrated into a single Chemistry LOE category value for each sampling location. It should be noted that the CA LRM and the CSI indices do not utilize all the same sediment chemistry constituents. While cadmium, dieldrin, trans nonachlor and 4,4'-DDT are solely utilized in the CA LRM calculation, gamma chlordane, total DDDs, total DDEs and total DDTs are solely utilized in the CSI calculation. All other target constituents are used in both indices. The first two columns of Table 3.4 indicate which of the indices utilizes each of the constituents.

California Logistic Regression Model

The CA LRM uses a logistic regression model to predict the probability of sediment toxicity based on sediment chemical constituent concentrations. The relationships between concentration and probability of toxicity have been established for all of the constituents used in the CA LRM (Bay *et al.* 2012). An example, for cadmium, is shown in Figure 3.1.

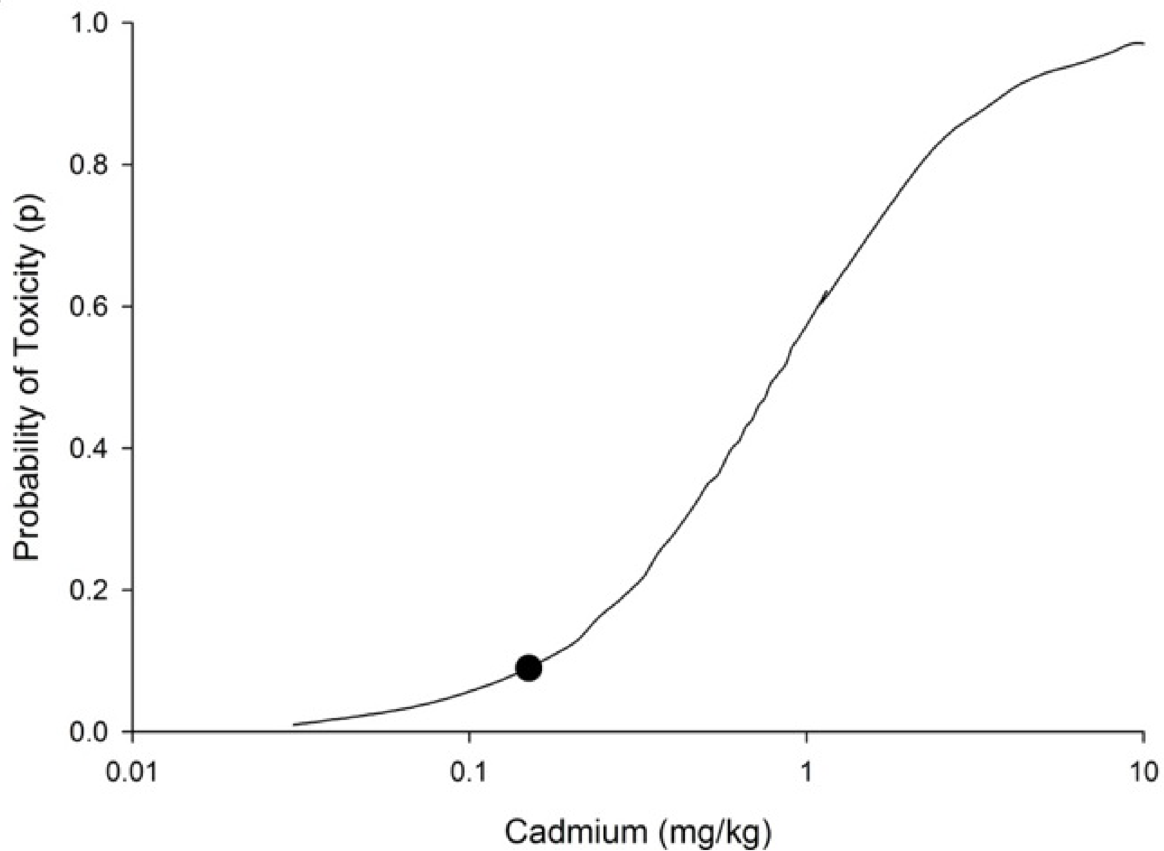


Figure 3.1. Logistic regression curve relating sediment cadmium concentration to probability of toxicity. The solid circle indicates the calculated probability of toxicity (> 0.1) based on a cadmium concentration of 0.15 mg/kg.

To determine the probability of toxicity for all the target constituents, the concentration data for each is entered in the following logistic regression equation:

$$p = e^{B0+B1\,(x)} / (1 + e^{B0+B1\,(x)})$$

Where: p = the probability of observing a toxic effect;

B0 = the intercept parameter (a *constant*, provided in Table 3.5);

B1 = the slope parameter (a *constant,* provided in Table 3.5); and,

x = the log of the concentration of the analyte of interest
(a *variable,* user-entered).

The result of each calculation is rounded to two decimal places.

Table 3.5 provides the values of B0 and B1 that should be used for the various sediment chemistry constituents to determine the CA LRM. It also shows the p values calculated for each target analyte given the data in Table 3.4.

Table 3.5. CA LRM parameters (constants B0 and B1) and p results (calculated) based on the data in Table 3.4.

Chemical	B0	B1	p value
Cadmium	0.2894	3.1764	0.09
Copper	-5.5931	2.5885	0.21
Lead	-4.7228	2.8404	0.40
Mercury	-0.0618	2.6837	0.58
Zinc	-5.1337	2.4205	0.25
HPAH	-8.1922	1.9995	0.15
LPAH	-6.8071	1.8827	0.09
Alpha Chlordane	-3.4080	4.4570	0.23
Dieldrin	-1.8344	2.5890	0.22
Trans Nonachlor	-4.2590	5.3135	0.10
PCBs, total	-4.4144	1.4837	0.08
4,4'-DDT	-3.5531	3.2621	0.09

Using the same logistic regression equation, the probability (p) of cadmium toxicity, based on data from Table 2.4 and parameters from Table 3.5, would be determined as follows:

$$p = e^{0.2894 + 3.1764 * \log(0.15)} / (1 + e^{0.2894 + 3.1764 * \log(0.15)})$$

$$p = e^{-2.328}/(1 + e^{-2.328})$$

$$p = 0.09749/1.09749$$

p = 0.09 (indicated by the dot in Figure 3.1)

The maximum p value among the target analytes from a given sediment sample is referred to as the “Pmax” value for that sample. The Pmax for the results in Table 3.5 corresponds to mercury. This Pmax value of **0.58** is compared to a set of response ranges to determine the CA LRM category for the sample. Table 3.6 provides these categories. A Pmax value of 0.58 places the sample in the Moderate Exposure category (> 0.49 to 0.66 ≤), which yields a category score of **3**. Thus 3 is the CA LRM result for the site in the example.

Table 3.6. Response ranges of Pmax for determination of the CA LRM category score.

Category	Range	Category Score
Minimal Exposure	< 0.33	1
Low Exposure	≥ 0.33 - 0.49 ≤	2
Moderate Exposure	> 0.49 - 0.66 ≤	3
High Exposure	> 0.66	4

Chemical Score Index

The CSI is calculated independently of the CA LRM and requires a four-step process. The first step involves comparing the concentration of each chemical constituent (e.g., the data in Table 3.4) to a series of concentration ranges that correspond to predicted benthic disturbance level (Ritter *et al.* 2012). Where the chemical constituent falls within these ranges determines the chemical exposure score (Table 3.7).

Table 3.7. Chemical concentration ranges for the chemical exposure categories used in the CSI calculation.

	Chemical Exposure Score			
Chemical Constituent	**1**	**2**	**3**	**4**
Copper (mg/kg dry wt.)	≤ 52.8	> 52.8 to ≤ 96.5	> 96.5 to ≤ 406	> 406
Lead (mg/kg dry wt.)	≤ 26.4	> 26.4 to ≤ 60.8	> 60.8 to ≤ 154	> 154
Mercury (mg/kg dry wt.)	≤ 0.09	> 0.09 to ≤ 0.45	> 0.45 to ≤ 2.18	> 2.18
Zinc (mg/kg dry wt.)	≤ 113	> 113 to ≤ 201	> 201 to ≤ 629	> 629
HPAH (µg/kg dry wt.)	≤ 313	> 313 to ≤ 1325	> 1325 to ≤ 9320	> 9320
LPAH (µg/kg dry wt.)	≤ 85.4	> 85.4 to ≤ 312	> 312 to ≤ 2471	> 2471
Alpha Chlordane (µg/kg dry wt.)	≤ 0.50	> 0.50 to ≤ 1.23	> 1.23 to ≤ 11.1	> 11.1
Gamma Chlordane (µg/kg dry wt.)	≤ 0.54	> 0.54 to ≤ 1.45	> 1.45 to ≤ 14.5	> 14.5
DDDs, total (µg/kg dry wt.)	≤ 0.77	> 0.77 to ≤ 3.56	> 3.56 to ≤ 26.37	> 26.37
DDEs, total (µg/kg dry wt.)	≤ 1.19	> 1.19 to ≤ 6.01	> 6.01 to ≤ 45.84	> 45.84
DDTs, total (µg/kg dry wt.)	≤ 0.61	> 0.61 to ≤ 2.79	> 2.79 to ≤ 34.27	> 34.27
PCBs, total (µg/kg dry wt.)	≤ 11.9	> 11.9 to ≤ 24.7	> 24.7 to ≤ 288	> 288

In the second step, the weighted score for each constituent is calculated by multiplying its score by its respective weighting factor, provided in Table 3.8.

$$CSI = \Sigma(w_i * S_i)/\Sigma w$$

Where: S_i = score for chemical i (from Table 3.7);

w_i = weight factor for chemical i (from 3rd column in Table 3.8); and

Σw = sum of all weights.

Table 3.8. Results of CSI calculations based on example dataset in Table 3.4.

Chemical	**Score (*determined* from Table 3.7)**	**Weight (a *constant*)**	**Weighted Score (*calculated*)**
Copper	1	100	100
Lead	2	88	176
Mercury	3	30	90
Zinc	1	98	98
HPAH	3	16	48
LPAH	2	5	10
Alpha Chlordane	3	55	165
Gamma Chlordane	3	58	174
DDDs, total	3	45	135
DDEs, total	2	33	66
DDTs, total	3	20	60
PCBs, total	2	55	110
Sum		603	1232
Weighted Mean (Weighted Score Sum/Weight Sum) = **2.04**			

The third step is to calculate the weighted mean score (CSI) by summing the weighted scores for all target analytes and dividing by the sum of all the weights (shown on the bottom of Table 3.8). If data are missing for any constituent, both the score and weight for that constituent become zero, thus adjusting both the sum of the weighted scores and sum of all weights accordingly.

The final part of the process is to compare the CSI value to a series of ranges to determine the CSI category. These ranges are provided in Table 3.9. The CSI value for the example data in Table 3.8 is **2.04**, which places it in the Low Exposure category from Table 3.9, yielding a category score of **2**. Thus 2 is the CSI result for the site in the example.

Table 3.9. CSI threshold ranges.

Category	Range	Category Score
Minimal Exposure	< 1.69	1
Low Exposure	≥ 1.69 - 2.33 ≤	2
Moderate Exposure	> 2.33 - 2.99 ≤	3
High Exposure	> 2.99	4

Integration of the Sediment Chemistry Indices

The final step in calculating the Chemistry LOE is to integrate the results for the two sediment chemistry indices: CA LRM and CSI. This is achieved by calculating the average of their two category scores. If the average falls between two response ranges, the value is rounded up to the next integer. The rounding methodology was specified by the SWRCB to provide a conservative estimate of the Chemistry LOE when the index results disagree. The numeric average can be also expressed as a descriptive category corresponding to the score. For the example data, the category score for the CA LRM was 3 and the category score for the CSI was 2. The average is 2.5, which rounds up to 3, yielding a Chemistry LOE category of Moderate Exposure.

Chapter 4: Benthic Community Composition

The composition of the benthic community constitutes an essential LOE for sediment quality assessment. The Benthic LOE is a direct measure of the effect that sediment contaminant exposure has on the benthic biota of California's bays and estuaries. Determination of the Benthic LOE is based on a series of community-based benthic condition indices. In the polyhaline and euhaline portions of Southern California and San Francisco Bay (i.e., Habitats C and D), a suite of 4 indices is used to calculate the Benthic LOE: 1) the Index of Biotic Integrity (IBI), 2) the Relative Benthic Index (RBI), 3) the Benthic Response Index (BRI), and 4) the River Invertebrate Prediction and Classification System (RIVPACS). In all other bay and estuarine habitats within California, the Multivariate-AMBI (M-AMBI) index can be used to calculate the Benthic LOE. This chapter includes computational tools for calculating the Benthic LOE category with all indices and provides an example of the step-by-step process for its determination.

Objectives

The goal of this chapter is to provide recommendations for laboratory processing, quality assurance (QA), quality control (QC), and data analysis procedures that are recommended for assessing the condition of soft bottom benthic macroinvertebrate communities of California's bays and estuaries. It is intended to supplement protocols presently used in California regarding methods that meet the requirements of the sediment quality assessment framework contained in the sediment quality objectives (SQO) policy.

Scope

This chapter describes laboratory procedures recommended for the processing of benthic infauna samples and data analysis methods for use in the California sediment quality objectives (CASQO) program. All key aspects of sample processing are described, including sample preservation, sorting, taxonomic analysis, quality assurance/quality control (QA/QC), and data analysis. A high level of detail regarding methods and benthic indices are included in this document as few step-by-step guidance documents are available.

Efficient sample sorting and accuracy in taxonomic identification are critical to obtaining high quality results. Species identification requires a high level of expertise by qualified taxonomists and there is always the potential for inaccurate results due to changes in nomenclature or subjective interpretation of diagnostic characteristics. Consequently, this chapter contains detailed recommendations for assuring the quality of sample processing. Example forms for recording the results of QA/QC activities are also provided in the appendices.

Sample Processing

Benthic sample processing in the laboratory includes the following tasks (Figure 4.1):

- Sample Preservation: The sample is washed free of formalin fixative and transferred into an alcohol solution for processing and storage.

- Sorting: Organisms are removed from sample debris, sorted into taxonomic groupings to facilitate subsequent taxonomic analysis, and sorting quality is evaluated and corrected if deficient.
- Taxonomic Analysis: Organisms in samples are identified and counted, voucher specimens are prepared to document identifications, and taxonomic analysis accuracy may be evaluated by reanalyzing selected samples.
- Data Entry: Taxonomic analysis and quality control results are recorded.
- Data analysis: The habitat type is determined, and the taxonomic analysis data are processed to determine the Benthic LOE category for each sampling site.

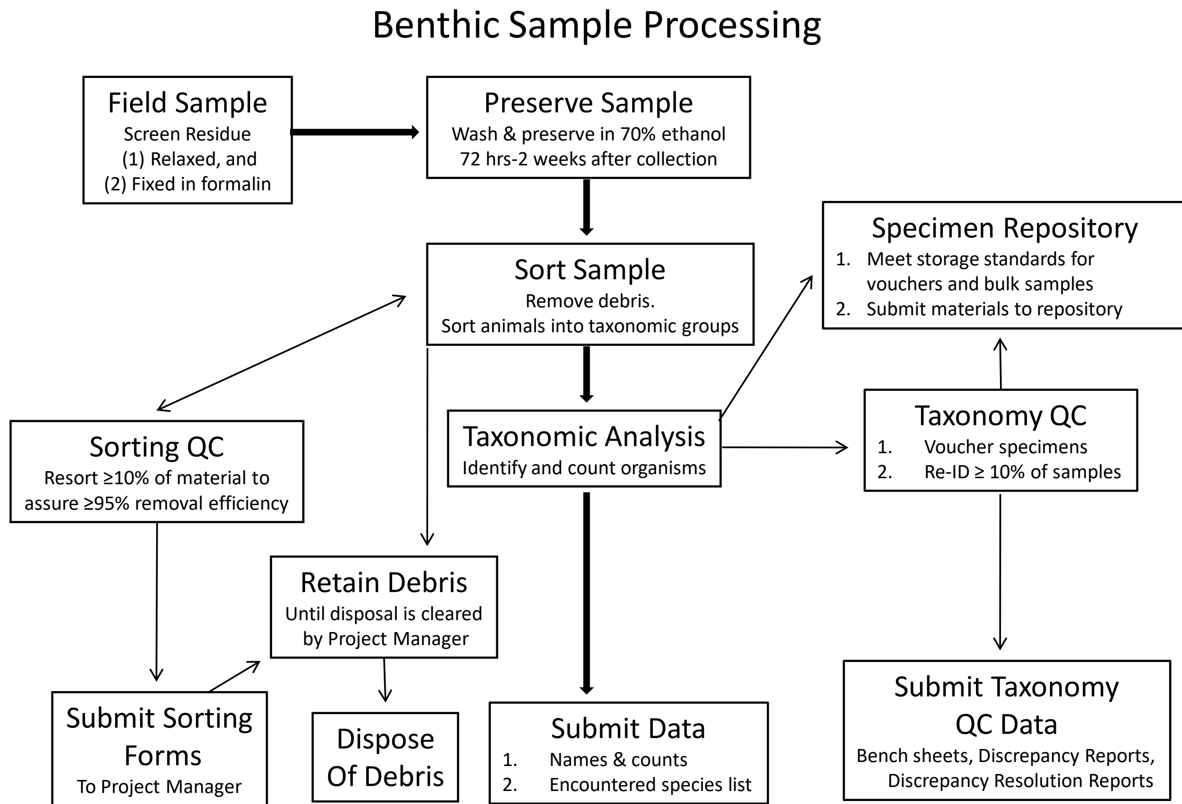


Figure 4.1. Overview of laboratory processing for benthic community samples.

Sample Preservation

Samples that are received from the field in formalin fixative must be washed and transferred to alcohol preservative. The removal of formalin is necessary for two reasons. Formaldehyde becomes increasingly acidic over time and prolonged exposure damages organisms with calcareous structures (e.g., shelled molluscs), which are often essential for accurate identifications. Secondly, formaldehyde is a noxious, potentially dangerous chemical. Replacing formaldehyde with ethanol makes subsequent sample handling safer. Other benefits of the washing process are the removal of excess silt from mud balls and fecal pellets that may have broken down during fixation and, in some cases, the opportunity to separate most of the organisms in a sample from inorganic debris using an elutriation process (defined below).

Samples fixed in formalin in the field should remain in formalin fixative for at least 72 hours, but no sample should remain in fixative for longer than two weeks because formalin will decalcify molluscs and echinoderms. Benthic community samples should be preserved in a 70% ethanol solution. Denatured alcohol and dyes for staining organisms are not recommended. The alcohol preservative should be buffered with marble chips, especially if the ethanol is produced by industrial distillation rather than fermentation. Ethanol is commonly purchased as a 95% ethanol solution. To prepare 1 L of 70% ethanol solution, 263 ml of purified water (e.g., filtered and de-ionized by reverse osmosis) is added to 737 ml of 95% ethanol. If samples contain a high percent of crustaceans, it is recommended to substitute some water with glycerin (e.g., 70% ethanol, 25% purified water, 5% glycerin) to help maintain exoskeleton shape.

Sample Sorting

Sorting is the process by which organisms in a benthic sample that were alive at time of collection are removed from the organic and inorganic residues (debris) that compose the sample and placed into broad taxonomic categories (taxonomic lots) for subsequent analysis by taxonomists. Sorting must be accurate and complete to assure the value of subsequent steps in the sample analysis process. Quality control procedures (see below) are used to assure that sorting accuracy and completeness meet data quality objectives (DQOs).

Several sorting techniques are used for the removal of benthic organisms from sediment. Commonly, a small amount of sample is placed in a Petri dish and each organism is systematically sorted and removed under a dissecting microscope using forceps. The “elutriation” or “floating” method is a technique that is effective when a sample is primarily coarse sand or highly organic. Inorganic material in the sample is separated from the lighter organic debris and organisms by the following elutriation process: After washing the formalin from the sample, spread the sample material out in a shallow pan or flat tray and cover with water. Gently agitate the sample by hand to allow the lighter fraction of debris and organisms to separate from the heavier material. The densest material settles to the bottom while the less dense material, such as organic material, arthropods, and other soft-bodied organisms, becomes suspended. The solution is then poured through the sieve and sorted. The denser material (e.g., sand grains and molluscs) is covered with water, so that it is more easily sorted and removed under a dissecting microscope. The water containing the lighter material should be decanted through a sieve, repeating the process several times until no more material is observed in the decanted water. Then the material in the decanted water is collected into a small sample container, topped with preservative, and returned to the original sample container along with the

balance of the sample material. The sample container should be filled with preservative and its lid tightly affixed. Both containers should be labeled properly with internal labels.

It is generally recommended that sorting be done in 70% ethanol, with care taken to assure that the sample being sorted is always fully covered with alcohol. It is not uncommon for Ophiuroidea to be removed from the ethanol and air dried to assist with identification. Organisms removed from the sample are sorted into taxonomic lots for subsequent taxonomic analysis. Remove all individual organisms and fragments from the sample with the exception of nematodes, foraminiferans and planktonic species or life stages. All fragments, such as decapod chelae and legs, should be placed in their respective taxa lots. The number and identity of taxa lots composing the sorted sample, the number of containers used if sample is split, and the time (to the nearest one-half hour) required to sort the sample should be recorded on the sorting record form (Appendix C).

Aggregate the taxa lots into one or more sample containers. It is generally recommended that each sample container and taxa lot be internally labeled with station name, sampling date and depth, and split number (if more than one container is used). Labels should be written in pencil or indelible ink on 100% rag-paper, poly-paper, or other paper suitable for permanent wet labels.

A breakdown of recommended taxonomic lots is provided in Table 4.1. The purpose of the taxonomic lots is to facilitate taxonomic analysis by project taxonomists, with each lot being analyzed by a single taxonomist. Therefore, the specifics of taxonomic lots may vary with the number of project taxonomists available and the details of their taxonomic expertise. In Southern California Bight Regional Monitoring Projects, taxonomic lots are usually the same as those into which identified and enumerated materials are stored (Table 4.1).

Table 4.1. Taxonomic lots for Southern California Bight regional monitoring projects.

Annelid Lots	Arthropod Lots	Mollusc Lots	Echinoderm Lots	Misc. Phyla Lots
Oligochaeta	Ostracoda	Bivalvia	Ophiuroidea	Cnidaria
Spionidae	Amphipoda	Gastropoda	Misc. Echinodermata	Nemertea
Cirratulidae	Isopoda	Misc. Mollusca		Other Phyla (a collective lot)
Misc. Polychaetes	Decapoda			
	Misc. Arthropoda			

Quality Control

Quality control of sorting is essential to assure the value of all the subsequent steps in the sample analysis process. A standard sorting form (Appendix C) is usually used for tracking the sample. It includes the name of the technician responsible, time required for sorting, comments, and re-sorting results. Re-sorting of samples is employed for QC purposes. It is good practice to have, at a minimum, 10 to 20% of all samples re-sorted to monitor sorter performance.

There are two recommended approaches used for re-sorting: the aliquot sample method, and the whole sample method. A laboratory may choose one of these two methods but, for consistency, a single method should be employed by a laboratory for all samples in a single project. The re-sort method used should be noted on the sorting form along with the re-sort results.

Whole Sample Method:

At least 10% of the samples processed by each sorter are completely re-sorted.

Aliquot Method:

A representative aliquot of at least 10% of the sample volume of every sample processed by each sorter is re-sorted.

Regardless of the method employed, an experienced sorter other than the original sorter conducts all re-sorting and percent sorting efficiency is calculated as follows:

Whole Sample Method:

$$\%Efficiency = 100 * [\#_{Original} / (\#_{Original} + \#_{Resort})]$$

Aliquot Method:

$$\%Efficiency = 100 * \{\#_{Original} / [\#_{Original} + (\#_{Resort} / \text{aliquot fraction})]\}$$

If sorting efficiency is greater than 95% (i.e., no more than 5% of the organisms in the original sample are missed), then no action is required. Sorting efficiencies below 95% initiate continuous monitoring of the underperforming technician. Failure to achieve 95% sorting efficiency initiates re-sorting of all samples previously sorted by that technician. Organisms found during re-sort should be included in the results from the sample. The calculated sorting efficiency is recorded on the sorting form for each sample that is re-sorted. The laboratory responsible for sorting should retain sample debris left after sorting until cleared for disposal. The debris should be properly labeled and preserved with 70% ethanol.

Taxonomic Analysis

The goal of taxonomic analysis is to identify accurately all organisms present in each sample to species level (or the lowest possible taxonomic level) and provide an accurate count of the organisms in each identified taxon.

Because of difficulties in the taxonomy and the lack of taxonomic expertise, exceptions to the goal of species-level identification are often established for a few groups of organisms. Examples are: Kinorhynchs are often only identified to phylum Kinorhyncha; in saline waters, Oligochaete annelids are often identified only to class Oligochaeta; Hirudinean annelids are often identified only to class Hirudinea; Podocopid ostracods are identified only to order Podocopida; and Harpacticoid copepods are identified only to order Harpacticoida. However, if the taxonomist is confident in more specific identification of these taxa (especially oligochaetes), we would recommend using a more refined taxonomic level.

Data for organisms that are incidental contaminants are not included in data analysis and should not be counted or included in project data. They are included as notes on the bench data sheets. For example, hard bottom epifaunal organisms such as barnacles occur incidentally in samples collected immediately adjacent to hard structures such as piers in harbors; their counts should not be included in the project data.

The numbers of organisms reported for a sample should include all organisms alive at the time of collection. Care should be taken to not count any individual more than once. Inevitably, samples contain fragments of organisms. It is recommended that fragments of bilaterally symmetrical organisms should be identified and counted only if the fragment includes the anterior end of the organism. Only fragments of radially symmetrical organisms (e.g., ophiuroids and anthozoans) containing the majority of the oral disk should be identified and counted. Care must be taken to avoid reporting empty mollusc shells or crustacean molts in the data.

Attached parasites and other epibionts should not be recorded or submitted in survey data but may be noted as present on the bench data sheet. Ectoparasites of fish that may be temporary members of the benthic community, such as cymothid isopods, are counted and reported in the data.

Nomenclature and orthography should follow the usage in the SQO species list on the *Sediment Quality Assessment Tools* page of the Southern California Coastal Water Research Project (SCCWRP) website (www.sccwrp.org) for purposes of calculating benthic condition assessment values. This list represents a consensus for standard usage of taxon names in the data used to develop SQOs and the Benthic LOE indices in southern California bays and estuaries and San Francisco Bay. These lists reflect the levels of identification used to calculate measures such as numbers of taxa and tolerance scores for benthic indices included in benthic line of evidence development. Compatibility and consistency of nomenclature is necessary to maintain the efficacy and intent of benthic indices and their condition thresholds within the context of SQOs. Taxonomic nomenclature, however, evolves with ongoing research. As such, present-day taxonomic standards will invariably differ from that of the SQO species list. To rectify these differences, the taxonomic standards of the present day will need to be "rolled back" to the standard used during the creation of the SQO species list.

Care should be taken in the rolling back of present-day taxonomic standards to that of the SQO species list and ideally should involve the participation of taxonomic experts familiar with the changes in nomenclature. Appendix E contains a table detailing changes in the names of taxa used in the calculation of Benthic LOE indices between the SQO species list and taxonomic standards of 2019. As detailed in the appendix, changes in name should be based upon agreement of both the Taxon and Authority fields. In addition to this resource, other sources of taxonomic standards, such as the most recent edition of the Southern California Association of Marine Invertebrate Taxonomists (SCAMIT) taxonomic listing, available at www.scamit.org, should be used to roll back taxon names because it lists synonyms that may have replaced names on the SQO species list.

Taxon (species) names in species abundance data tables (see data entry section that follows) follow special rules and are standardized in spelling and form. Because the "species" field is one of the key fields for defining a unique record, precision is required. To minimize the problem of variants, standard spelling and formation for names is based on names in the SQO species list on the *Sediment Quality Assessment Tools* page of the SCCWRP website (www.sccwrp.org), and the most recent edition of the SCAMIT taxonomic listing, available at www.scamit.org. The name used to represent a taxon generally should be that listed in the species list and the species field should contain only genus and species names free of any punctuation, including periods, commas, and quotation marks. Descriptors such as "juvenile" or "fragment" are reserved for the comments field. If it is desired to separate adults and juveniles of a species, the number of

juveniles can be carried in the comments field, but the abundance number should reflect the total number of animals of that species in that sample. The recommendations for levels of taxonomic resolution specified in the chapter providing guidance for SQO taxonomic analysis are also relevant. Samples with no organisms are recorded with the species name "No organisms present" and a blank (missing) or zero abundance to clearly indicate that the sample was collected but no organisms were present.

Temporary "in-house" provisional names are erected for specimens that a taxonomist considers to be distinctive but cannot match with an existing description. Provisional names should be resolved prior to data submission and analysis by assigning a valid name (binomen acceptable to the International Commission on Zoological Nomenclature (ICZN) or by documenting the characteristics of the provisional taxon on a voucher sheet that meets the Voucher Sheet standards of the SCAMIT.

Taxonomists identifying and enumerating benthic macrofauna in samples should also be aware of and utilize "exclude" and "voucher" notations. The "exclude" field provides an aid to data analyses that involve calculating numbers of taxa by presenting the taxonomist's recommendation that the reported taxon be excluded from counts of the number of taxa in the sample, as it may not be distinct. This is useful when the taxon in question is already included in another row. Specifically, the "exclude" annotation is employed when three conditions co-exist:

- The identification is not at the species-level (e.g., Pleustidae or *Polydora* sp.).
- The reported taxon is represented in the sample by other members of its taxon, which have been identified at lower levels (e.g., *Streblospio websteri* and *Streblospio* sp in the same sample).
- The taxonomist cannot determine if the specimen is distinct from other members of its taxon in the sample.

Taxonomists should make this evaluation during sample analysis (i.e., by an annotation on the bench sheet). It cannot be effectively applied after the fact, because there is no way of determining later whether the third criterion for use was met. An example would be recommending that organisms identified only as Spionidae be excluded because other organisms were identified as *Pseudopolydora paucibranchiata*, which is a spionid polychaete. The rationale for this is that, although the spionids should count when computing abundance, they should be excluded from calculations of numbers of taxa unless they are clearly not *P. paucibranchiata*.

Voucher counts document removal of specimens from a sample and this notation on the bench sheet is essential for integrity of the quality control and assessment process. Removal of organisms for voucher collections without annotation confuses the resolution of discrepancies during quality control reanalysis and leads to overstatement of error rates.

Quality Control

The goal of taxonomic analysis for macrofaunal samples is species-level identification of all macrobenthic organisms collected, and an accurate count for each species. Establishing voucher collections and reanalysis of a subset are two control activities that are very strongly

recommended for every study. Quality assurance activities and general best practices should also include participation of the responsible taxonomists in regional or statewide taxonomic organizations (such as SCAMIT) or taxonomic workshops.

Voucher Collections

The purpose of a voucher collection is to provide good quality specimens exemplifying project taxonomists' usage of each name in the data. In cases where questions about nomenclature arise, or a portion or the entirety of a taxon is subsequently synonymized, examination of the vouchers may resolve uncertainties.

Each voucher container should contain an internal label bearing the complete taxon name, author, and date. Only glass containers are used for the storage of voucher material, unless specimens are inappropriate for wet storage. Within the voucher container, each specimen lot should be contained within a shell vial closed with a cotton stopper. Shell vials should have a minimum capacity of one-half dram. Specimens too large to be contained in shell vials may be stored in jars. Each voucher lot contains an internal label bearing the taxon name, station name of sample from which the specimen(s) was removed, a count of the number of specimens in the lot, the analytical laboratory's designation, and the identifying taxonomist's initials. Labels should be written in pencil or indelible ink on 100% rag-paper, poly-paper, or other paper suitable for permanent wet labels.

Subordinate to project voucher requirements, individual labs or taxonomists may remove a limited number of specimens for their own voucher collections. Any and all unique specimens should be included in project, rather than individual, voucher collections.

Sample Reanalysis

Best practices for providing data quality control include an assessment of the laboratory's taxonomic discrimination, taxonomic accuracy, and count accuracy by re-analysis of a subset of samples by independent taxonomists. Discrepancies between the original and quality control taxonomists are resolved by comparing results of the two sets of identifications.

A minimum of 10% of the samples processed by a laboratory should be re-analyzed. Samples for re-identification are selected at random after the initial identification is complete, ensuring that there is no prior knowledge of the identity of reanalysis samples. Re-identification should be conducted by taxonomists other than those who originally analyzed the samples and ideally from a different laboratory. Quality control taxonomists should not have access to the original taxonomic analysis results.

After re-analysis is complete, quality control taxonomists are provided the original results, which are compared with the quality control re-analysis results, and all discrepancies are listed on a Discrepancy Report (Appendix C). The discrepancy classification and resolution codes to be used are presented in Appendix C. In addition to discrepancy classification and resolution codes, error types (true, random, non-error), and recommended QC remedial action (training, review best practices) are presented for each resolution code. The naming convention discrepancy code refers to differences in name usage and/or spelling. The variation in level of expertise resolution code notes differences in knowledge or standard practice between taxonomists when addressing

especially difficult taxonomic groups or damaged/juvenile specimens. Errors in the QC data, while important to note for feedback to the QC taxonomists, do not affect the resolved final data set and thus do not require changes.

Taxonomic discrepancies should be discussed, and final resolutions determined, through meetings between primary and QC taxonomists. The cause and resolution of discrepancies should be reported on the Discrepancy Report using discrepancy classification and resolution codes. While completion of this spreadsheet is the responsibility of the QC laboratory, both labs must work together to reach agreement. This process may include consulting additional experts, if necessary. Once resolution and explanation of all discrepancies is complete, the Discrepancy Resolution Report, copies of both laboratories' bench sheets, and the Discrepancy Report are used to calculate the percent error of the original laboratory's analysis. The percent error should be calculated for three aspects of taxonomic analysis: Accuracy of Taxonomic Discrimination ($\%Err_{\#\,Tax}$), Accuracy of Counting ($\%Err_{\#\,Count}$), and taxon-specific Accuracy of Identification, weighted by abundance ($\%Err_{ID}$) as follows:

$$\%Err_{\#\,Tax} = \{1-[(Taxa_{Orig} - Taxa_{Res})/Taxa_{Res}]\}*100$$

$$\%Err_{\#\,Count} = \{1-[(Total\ Abundance_{Orig} - Total\ Abundance_{Res})/Abundance_{Res}]\}*100$$

$$\%Err_{ID} = [1-(Mis\text{-}ID\text{'}d\ Individuals_{Orig} / Number\ of\ Individuals_{Res})]*100$$

The first two aspects provide measures of data quality as relates to parameters such as species richness, abundance, and diversity. The third aspect, identification accuracy, is expressed as percent error in identification of individual taxa. It provides a measure of data quality as a representation of community composition. The calculations consider only errors in the original analysis.

The results are reported on an Infaunal Identification and Enumeration Accuracy Report (Appendix C).

Based upon the results of data quality assessment, a DQO of 10%, representing the maximum deviation from the "true" value, is recommended for number of taxa, total number of organisms, and identification accuracy. Each laboratory should strive to avoid exceeding this level of error and we would recommend rejection of data from all samples associated with the re-ID sample that failed to meet the designated DQOs. Systematic re-identification of all samples from that taxonomist and taxonomy lab should be required before data are considered acceptable for any subsequent SQO analysis.

Data Entry

The taxonomic analysis results are usually stored in a species abundance data table that documents the numerical presence of all infaunal animals collected in each sample. Each row presents the abundance of a single species in a sample. The table contains as many rows as the sum of the number of taxa in all the samples. Details of the information usually included in the columns of a species abundance table are presented in Table 4.2. Data formats for calculation of M-AMBI scores differs slightly from that of the BRI, IBI, RBI, and RIVPACS indices, and are detailed below in the *Other California Estuaries and Embayments* section.

Table 4.2. Example table structure for species abundance data.

Field (Column) Name	Field Type	Field Required?	Description
StationID	Text	Yes	Station name
SampleID	Text	No	Internal laboratory sample identification
Replicate	Text	Yes	The sequential number of the grab
Sample Date	Date/Time	Yes	The sample collection date
Species	Text	Yes	The taxon name
Qualifier	Text	No	Abundance qualifier from an established list (e.g., colonial organisms)
Abundance	Number	Yes	Number of individuals (0 for colonies)
Exclude	Yes/No	Yes	Flag to exclude when counting number of taxa
Lab Code	Text	Yes	Laboratory identification
Screen Size	Text	Yes	Sieve or screen size used to process samples - usually 1.0 or 0.5
Screen Size Units	Text	Yes	Usually millimeters (mm)
Voucher	Number	No	Number of animals from this sample that were vouchered
Comments	Text	No	Comments

Taxon (species) names in species abundance data tables follow special rules and are standardized in spelling and form. Because the "*Species*" field is a key field for defining unique records, exactitude is required. To minimize the problem of variants, standard spellings are on the SQO species list on the *Sediment Quality Assessment Tools* page of the SCCWRP website (www.sccwrp.org), and the most recent version of the SCAMIT's taxonomic listing, available at www.scamit.org. Additional details about the contents of the species name field are presented in the taxonomic analysis section.

The "*Exclude*" field provides an aid to data analysis for calculating numbers of taxa by documenting the taxonomist's recommendation that a taxon be excluded from taxon counts because it is already included in another row. Voucher notations document removal of specimens from a sample and are essential to the quality control and assessment process. Refer to the taxonomic analysis section of this chapter for more detailed information about the "exclude" and "voucher" notations.

Data Analysis to Determine Benthic Invertebrate Community Condition

Introduction

The benthic fauna of a given waterbody are useful in assessing the exposure of that location of toxic chemicals and other types of stressors because they are a direct measure of the aquatic life

beneficial uses and the community composition changes in relatively predictable ways to disturbance. These changes are measured with the taxonomic data derived from a benthic sample and are in turn quantified using condition assessment indices. These indices translate the shifts in taxonomic composition and abundance into ecologically and managerially meaningful values by comparing them to benchmarks of habitat condition.

However, in addition to anthropogenic disturbance, benthic community composition will also reflect differences in environmental factors like salinity, temperature, sediment composition, water depth, or tidal inundation. Furthermore, given the nature of their larval dispersal and relatively sessile nature as adults, biogeography will also influence the taxonomic composition of benthic fauna. As such, when using benthic fauna to evaluate exposure to anthropogenic disturbance it is vital to be able to account for change in community structure due to natural factors. In naturally heterogeneous habitats, like estuaries and coastal embayments, this is even more critical, as fauna indicative of disturbance in higher salinities may be "normal" taxa in lower/more variable salinities.

Within the SQO program, it was decided to control for the multiple factors that can influence benthic community composition by dividing the subtidal sediments of California's estuaries and embayments into discrete habitats. Habitats were determined from differences in benthic community composition at clean, undisturbed locations from different geographies, salinity regimes, and sediment types (see Ranasinghe et al. 2012). This approach minimizes community variation due to environmental factors and biogeography within a designated habitat and enabled the development of indices that could quantify the changes in community structure due to anthropogenic disturbance.

In the end, three broad classes of estuary/embayment were distinguished for application of benthic condition assessment tools and determination of a Benthic LOE: 1. High salinity (>27 PSU) marine (polyhaline or euhaline) waters in Southern California (i.e., Habitat C); 2. High salinity (> 27 PSU) polyhaline waters within San Francisco Bay (i.e., Habitat D); and 3. Other habitats, including low salinity waters of Southern California and San Francisco Bay, as well as all other subtidal estuaries and embayments outside of Southern California and San Francisco Bay (i.e., Habitats E, F, G, and H). Within each of these habitats, a suite of benthic assessment tools was investigated and the following sections detail the application of these tools in each location. Details on the development and testing of these different tools can be found in Ranasinghe et al. 2009, Gillett et al. 2015, Pelletier et al. 2018, and Gillett et al. 2019. Irrespective of their component parts, each of the benthic indices uniformly evaluates benthic condition of a sample in one of four categories:

- Reference: A community that would occur at a reference site for that habitat
- Slight Disturbance: A community that may exhibit some indication of stress, but is within measurement variability of reference condition
- Moderate Disturbance: A community that exhibits clear evidence of physical, chemical, natural, or anthropogenic stress
- High Disturbance: A community exhibiting a high magnitude of stress

Briefly, the steps necessary to determine the benthic community condition for a sample are:

1. **Data Preparation.** The raw data needed for the analyses include the abundance of each species (or lowest possible identification level) and station depth, latitude, longitude, and salinity of the overlying water. Each taxon should be identified to the appropriate level in keeping with the benthic macrofauna species list for the relevant habitat.

2. **Identification of Benthic Habitat Type.** The benthic habitat that was sampled must be determined in order to select appropriate benthic indices. A key to identify the habitat type from physical habitat factors and a table of dominant species, to verify that the assemblage corresponds with these factors, are provided in Appendix D.

3. **Calculation of benthic indices and determination of condition category.** The calculated benthic index values are compared to response ranges for determination of condition categories.

Southern California Marine Bays

Research in the polyhaline and euhaline portions of California embayments has shown that the use of a combination of benthic indices provides a more accurate description of benthic invertebrate community condition than does the use of a single index (Ranasinghe *et al.* 2009). This section describes the steps necessary to calculate four benthic indices and their thresholds for Southern California marine bays (Habitat C):

- Index of Biotic Integrity (IBI).
- Relative Benthic Index (RBI).
- Benthic Response Index (BRI).
- River Invertebrate Prediction and Classification System (RIVPACS).

Table 4.3 presents details of the metrics included for calculation of index values in Southern California Marine Bays. Instructions for calculating each of the indices and descriptions of the species list variables follow. Calculation of metrics should be based upon data sets with the taxonomic nomenclature from the time of collection adjusted to match that of the SQO species list, as suggested in Appendix E.

Table 4.3. Benthic indicator metrics in Southern California Marine Bays. Asterisks indicate metrics included in both the RBI and IBI.

Index	Metric	Use
IBI	Total number of taxa*	All taxa
	Number of mollusc taxa*	Molluscs
	Notomastus sp. abundance	*Notomastus* sp
	Abundance percentage of sensitive taxa	IBISensitive = S
RBI	Total number of taxa*	All taxa
	Number of mollusc taxa*	Molluscs
	Number of crustacean taxa	Crustaceans
	Number of crustacean individuals	Crustaceans
	Abundance of *Monocorophium insidiosum*	*Monocorophium insidiosum*
	Abundance of *Asthenothaerus diegensis*	*Asthenothaerus diegensis*
	Abundance of *Goniada littorea*	*Goniada littorea*
	Presence of *Capitella capitata* complex	*Capitella capitata* complex
	Presence of Oligochaeta	Oligochaeta
BRI	Abundance weighted average tolerance score	ToleranceScore
RIVPACS	Observed to expected (O/E) ratio for number of RIVPACS reference taxa	Instructions for calculating O/E Ratio using the SCCWRP website (Appendix A) or SAS Software (Appendix B)

Index of Biotic Integrity (IBI) and IBI condition category

The IBI compares the values of four different metrics to the ranges expected under reference conditions. Each metric that is outside of the reference range increases the IBI score by one. Therefore, if all four metrics were inside the reference range, the score would be 0. Conversely, if all four were outside the reference range, the value would be 4.

The data needed to calculate the IBI are the total number of taxa, number of mollusc taxa, abundance of *Notomastus* sp., and number of sensitive taxa (Table 4.3). The total number of taxa, and abundance of *Notomastus* sp. can be obtained directly from the data. The number of mollusc taxa should be limited to those taxa designated as molluscs on the SQO species list for Southern California Marine Bays. Other molluscs in the data should not be counted for this metric. The list of sensitive species should be based on the species list for Southern California Marine Bays and the percentage of sensitive taxa present is calculated as:

% sensitive taxa= (number of sensitive taxa/total number of taxa) * 100

The value for each metric is then compared to a reference range for that metric (Table 4.4). The IBI score is set to zero before comparison to the reference range. For each metric that is out of the reference range (above or below), the IBI score goes up by one.

Table 4.4. Reference ranges for IBI metrics in Southern California Marine Bays.

Metric	Reference Range
Total Number of Taxa	13 - 99
Number of Mollusc Taxa	2 - 25
Abundance of *Notomastus sp.*	0 - 59
Percentage of Sensitive Taxa	19 - 47.1

The IBI score is then compared to condition category response ranges (Table 4.5) in order to determine the IBI category and score.

Table 4.5. IBI category response ranges for Southern California Marine Bays.

IBI Score	Category	Category Score
0	Reference	1
1	Low Disturbance	2
2	Moderate Disturbance	3
3 or 4	High Disturbance	4

Relative Benthic Index (RBI) and RBI Condition Category

The RBI is the weighted sum of: 1) four community metrics related to biodiversity (total number of taxa, number of crustacean taxa, abundance of crustacean individuals, and number of mollusc taxa); 2) abundances of three positive indicator taxa; and 3) the presence of two negative indicator species.

The data needed to calculate the RBI are: total number of taxa, number of mollusc taxa, number of crustacean taxa, number of crustacean individuals, number of individuals of *Monocorophium insidiosum*, *Asthenothaerus diegensis*, and *Goniada littorea*, and the presence of *Capitella capitata* complex and Oligochaeta. Calculation of the number of mollusc taxa, crustacean taxa, and abundance of Crustacea should be limited to those taxa designated as molluscs or crustaceans on the SQO species list for Southern California Marine Bays. Other molluscs and crustaceans in the data should not be counted for these metrics.

The first step is to normalize the values for the benthic community metrics relative to maxima for the data used to develop the RBI for the Southern California Marine Bays habitat, to produce values relative to the maxima that are referred to as scaled values. The scaled value calculations use the following formulae:

Total number of taxa/99

Number of mollusc taxa/28

Number of crustacean taxa/29

Abundance of Crustacea/1693

The next step is to calculate the Taxa Richness Weighted Value (TWV) from the scaled values by the equation:

TWV = Scaled total number of taxa + Scaled number of mollusc taxa + Scaled number of crustacean taxa + (0.25 * Scaled abundance of Crustacea)

Next, the value for the two negative indicator taxa (NIT) is calculated. The two negative indicator taxa are *Capitella capitata* complex and Oligochaeta. For each of these taxa that are present, in any abundance whatsoever, the NIT is decreased by 0.1. Therefore, if neither were found the NIT = 0, if both are found the NIT = -0.2.

The next step is to calculate the value for the three positive indicator taxa (PIT). The positive indicator taxa are *Monocorophium insidiosum*, *Asthenothaerus diegensis*, and *Goniada littorea.* First, the PIT value is calculated for each species using the following equations:

$$\frac{\sqrt[4]{\textit{Monocorophium insidiosum} \text{ abundance}}}{\sqrt[4]{473}}$$

$$\frac{\sqrt[4]{\textit{Asthenothaerus diegensis} \text{ abundance}}}{\sqrt[4]{27}}$$

$$\frac{\sqrt[4]{\textit{Goniada littorea} \text{ abundance}}}{\sqrt[4]{15}}$$

The three species PIT values are then summed to calculate the PIT value for the sample. If none of the three species is present, then the sample PIT = 0.

The next step is to calculate the Raw RBI:

Raw RBI = TWV + NIT + (2 * PIT)

The final calculation is for the RBI Score, normalizing the Raw RBI by the minimum and maximum Raw RBI values in the index development data:

RBI Score = (Raw RBI - 0.03)/4.69

The last step in the RBI process is to compare the RBI Score to a set of response ranges to determine the RBI category (Table 4.6).

Table 4.6. RBI category response ranges for Southern California Marine Bays.

RBI Score	Category	Category Score
> 0.27	Reference	1
0.17 to 0.27	Low Disturbance	2
0.09 to 0.16	Moderate Disturbance	3
< 0.09	High Disturbance	4

Benthic Response Index (BRI) and BRI Condition Category

The BRI is the abundance weighted pollution tolerance score of the organisms present in a benthic sample. The higher the BRI score, the more degraded the benthic community represented by the sample.

Two types of data are needed to calculate the BRI, the abundance of each species and its pollution tolerance score, P. For most taxa present in this assemblage P values are available. Only species for which P values are available are used in the BRI calculations. P values should be obtained for the appropriate habitat and from the most up-to-date list available.

The first step in the BRI calculation is to compute the 4th root of the abundance of each taxon in the sample for which P values are available. The next step is to multiply the 4th root abundance value by the P value, for each taxon.

Next, separately sum all the 4th roots of the abundances and all of the products of the 4th roots of abundance and P values. Taxa that lack P values are not included in either sum.

Putting this all together into a single equation to calculate the BRI score yields:

$$\frac{\sum\left(\sqrt[4]{Abundance}\right)\times P}{\sum \sqrt[4]{Abundance}}$$

The last step is to compare the BRI score to BRI response ranges in Table 4.7 to determine the BRI category and category score.

Table 4.7. BRI category response ranges for Southern California Marine Bays.

BRI Score	Category	Category Score
< 39.96	Reference	1
39.96 to 49.14	Low Disturbance	2
49.15 to 73.26	Moderate Disturbance	3
> 73.26	High Disturbance	4

River Invertebrate Prediction and Classification System (RIVPACS) Index and RIVPACS Condition Category

The RIVPACS index calculates the number of reference taxa present in the test sample (observed or "O") and compares it to the number expected to be present ("E") in a reference sample from the same habitat. Calculation of the RIVPACS score is a three-step process. The first step consists of determining the probability of the test sample belonging to twelve Southern California Marine Bays reference sample groups. This determination is based on the sampling station's bottom depth, latitude, and longitude, using a complex linear discriminant function.

The second step is determining, for each sample, the identity and expected number of reference species, based on the probabilities of group membership calculated in Step 1 and the distribution of reference species in each group. In the final step, the number of reference species observed in the sample is counted, the observed/expected (O/E) RIVPACS score calculated and compared to the response ranges in Table 4.8 to determine the RIVPACS category and category score.

Table 4.8. RIVPACS category response ranges for Southern California Marine Bays.

RIVPACS Score	Category	Category Score
> 0.90 to < 1.10	Reference	1
0.75 to 0.90 or 1.10 to 1.25	Low Disturbance	2
0.33 to 0.74 or > 1.25	Moderate Disturbance	3
< 0.33	High Disturbance	4

Because of the complexity of the RIVPACS calculations, computer programs are used to determine the O/E values. Detailed instructions for calculating RIVPACS O/E values by two computer programs are provided in Appendices A and B. Appendix A contains directions for using a web-based tool on the SCCWRP website. Appendix B covers instructions for calculating RIVPACS O/E values using the Statistical Analysis System (SAS). The SAS programs calculate RIVPACS O/E values and condition categories but require availability of the SAS software. The SCCWRP tool is freely available with less rigid data formatting requirements.

Species list contents

The Southern California Marine Bays species list is provided in a spreadsheet that can be accessed from the SCCWRP website (www.sccwrp.org) under the Sediment Quality Assessment Tools section of the Sediment Quality research area. The contents of each column in the spreadsheet are described in Table 4.9.

Table 4.9. Southern California Marine Bays species list contents.

Column	Header	Contents
1	TaxonName	Taxon name
2	Phylum	Taxonomic phylum
3	Class	Taxonomic class
4	Order	Taxonomic order
5	Family	Taxonomic family
6	IBISensitive	When present, “S” indicates a taxon considered sensitive for calculation of the SoCal IBI
7	Mollusc	When present, “Mollusc” indicates molluscan taxa for RBI and IBI calculations
8	Crustacean	When present, “Crustacean” indicates crustacean taxa for RBI calculations
9	Tolerance Score	When present, values are tolerance scores for BRI calculation
10	RivColHead	When present, in the abundance data file submitted for RIVPACS calculations to the Utah State University web site, this exact text is used as the column header for abundance data for this taxon
11	RivColNo	When present, in the abundance data file submitted for RIVPACS calculations to the Utah State University web site, this is the column number containing abundances for this taxon.
12	SpeciesLevel	When present, “Drop” in this column indicates that abundances of this taxon are included in index calculations, but it is not included for counting numbers of taxa because lower taxonomic level entries in this taxon are also present.

San Francisco Bay Polyhaline

Table 4.10 presents details of the metrics included for calculation of index values in San Francisco Bay Polyhaline. Instructions for calculating each of the indices and descriptions of the species list variables follow. Calculation of metrics should be based upon data sets with the taxonomic nomenclature from the time of collection adjusted to match that of the SQO species, list as suggested in Appendix E.

Table 4.10. Benthic indicator metrics in San Francisco Bay Polyhaline. Asterisks indicate metrics included in both the RBI and IBI.

Index	Metric	Use
IBI	Total number of taxa*	All taxa
	Number of amphipod taxa	Amphipods
	Total abundance	All taxa
	Abundance of *Capitella capitata* complex	*Capitella capitata* Cmplx
RBI	Total number of taxa*	All taxa
	Number of mollusc taxa	Molluscs
	Number of crustacean taxa	Crustaceans
	Number of crustacean individuals	Crustaceans
	Abundance of *Sinocorophium heteroceratum*	*Sinocorophium heteroceratum*
	Abundance of *Rochefortia* spp.	*Rochefortia* spp.
	Abundance of *Prionospio (Minuspio) lighti*	*Prionospio (Minuspio) lighti*
	Presence of *Capitella capitata* complex	*Capitella capitata* Cmplx
	Presence of Oligochaeta	Oligochaeta
BRI	Abundance weighted average tolerance score	ToleranceScore
RIVPACS	Observed to expected ratio for number of RIVPACS reference taxa.	Instructions for calculating O/E Ratio using the SCCWRP website (Appendix A) or SAS Software (Appendix B).

Index of Biotic Integrity (IBI) and IBI Condition Category

The IBI compares the values of four different metrics to the ranges expected under reference conditions. Each metric that is outside of the reference range increases the IBI score by one. Therefore, if all four metrics were inside the reference range, the score would be 0. Conversely, if all four were outside the reference range, the value would be 4.

The data needed to calculate the IBI are the total number of taxa, number of amphipod taxa, total abundance, and abundance of *Capitella capitata* complex (Table 4.10). The value for each metric is then compared to a reference range for that metric (Table 4.11). The number of amphipod taxa

should be limited those taxa designated as amphipods on the SQO species list for San Francisco Bay Polyhaline. Other amphipods in the data should not be counted for this metric. The IBI score is set to zero before comparison to the reference ranges. For each metric that is out of the reference range (above or below), the IBI score goes up by one.

Table 4.11. Reference ranges for IBI metrics in San Francisco Bay Polyhaline.

Metric	Reference Range
Total Number of Taxa	21 - 66
Number of Amphipod Taxa	2 - 11
Total Abundance	97 - 2931
Abundance of *Capitella capitata* complex	0 - 13

The IBI score is then compared to condition category response ranges (Table 4.12) in order to determine the IBI category and score.

Table 4.12. IBI category response ranges for San Francisco Bay Polyhaline.

IBI Score	Category	Category Score
0 or 1	Reference	1
2	Low Disturbance	2
3	Moderate Disturbance	3
4	High Disturbance	4

Relative Benthic Index (RBI) and RBI Condition Category

The RBI is the weighted sum of: 1) four community metrics related to biodiversity (total number of taxa, number of crustacean taxa, abundance of crustacean individuals, and number of mollusc taxa); 2) abundances of three positive indicator taxa; and 3) the presence of two negative indicator species.

The data needed to calculate the RBI are: total number of taxa, number of mollusc taxa, number of crustacean taxa, number of crustacean individuals, number of individuals of *Sinocorophium heteroceratum*, the genus *Rochefortia*, and *Prionospio (Minuspio) lighti*, and the presence of *Capitella capitata* complex and Oligochaeta. Calculation of the number of mollusc taxa, crustacean taxa, and abundance of Crustacea, should be limited to those taxa designated as molluscs or crustaceans on the SQO species list for San Francisco Bay Polyhaline. Other molluscs and crustaceans in the data should not be counted for these metrics.

The first step is to normalize the values for the benthic community metrics relative to maxima for the data used to develop the RBI for the San Francisco Bay Polyhaline habitat. This produces values relative to the maxima that are referred to as scaled values. The scaled value calculations use the following formulae:

Total number of taxa/55

Number of mollusc taxa/13

Number of crustacean taxa/17

Abundance of Crustacea/17237

The next step is to calculate the Taxa Richness Weighted Value (TWV) from the scaled values by the equation:

TWV = Scaled total number of taxa + Scaled number of mollusc taxa + Scaled number of crustacean taxa + (0.25 * Scaled abundance of Crustacea)

Next, the value for the two Negative Indicator Taxa (NIT) is calculated. The two negative indicator taxa are *Capitella capitata* complex and Oligochaeta. For each of these taxa that are present, in any abundance whatsoever, the NIT is decreased by 0.1. Therefore, if neither were found the NIT = 0, if both are found the NIT = -0.2.

The next step is to calculate the value for the three Positive Indicator Taxa (PIT). The positive indicator taxa are *Sinocorophium heteroceratum*, *Rochefortia* spp, and *Prionospio (Minuspio) lighti.* First, the PIT value is calculated for each species using the following equations:

$$\frac{\sqrt[4]{Sinocorophium\ heteroceratum\ \text{abundance}}}{\sqrt[4]{1878}}$$

$$\frac{\sqrt[4]{Rochefortia\ \text{spp. abundance}}}{\sqrt[4]{105}}$$

$$\frac{\sqrt[4]{Prionospio\ (Minuspio)\ lighti\ \text{abundance}}}{\sqrt[4]{17}}$$

The three species PIT values are then summed to calculate the PIT value for the sample. If none of the three species is present, then the sample PIT = 0.

The next step is to calculate the Raw RBI:

Raw RBI = TWV + NIT + (2 * PIT)

The final calculation is for the RBI Score, normalizing the Raw RBI by the minimum and maximum Raw RBI values in the index development data:

RBI Score = (Raw RBI - 0.00)/6.88

The last step in the RBI process is to compare the RBI Score to a set of response ranges to determine the RBI category (Table 4.13).

Table 4.13. RBI category response ranges for San Francisco Bay Polyhaline.

RBI Score	Category	Category Score
> 0.43	Reference	1
0.30 -to 0.43	Low Disturbance	2
0.20 to 0.29	Moderate Disturbance	3
< 0.20	High Disturbance	4

Benthic Response Index (BRI) and BRI Condition Category

The BRI is the abundance weighted pollution tolerance score of the organisms present in a benthic sample. The higher the BRI score, the more degraded the benthic community represented by the sample.

Two types of data are needed to calculate the BRI, the abundance of each species and its pollution tolerance score, P. P values are available for most species present in the assemblage. Only species for which P values are available are used in the BRI calculations. P values should be obtained for the appropriate habitat and from the most up-to-date list available.

The first step in the BRI calculation is to compute the 4th root of the abundance of each taxon in the sample for which P values are available. The next step is to multiply the 4th root abundance value by the P value, for each taxon.

Next, separately sum all of the 4th roots of the abundances and all of the products of the 4th roots of abundance and P values. Taxa that lack P values are not included in either sum.

The next step is to calculate the BRI score as:

$$\frac{\sum\left(\sqrt[4]{Abundance}\right)\times P}{\sum \sqrt[4]{Abundance}}$$

The last step is to compare the BRI score to BRI response ranges in Table 4.14 to determine the BRI category and category score.

Table 4.14. BRI category response ranges for San Francisco Bay Polyhaline.

BRI Score	Category	Category Score
< 22.28	Reference	1
22.28 to 33.37	Low Disturbance	2
33.38 to 82.08	Moderate Disturbance	3
> 82.08	High Disturbance	4

River Invertebrate Prediction and Classification System (RIVPACS) Index and RIVPACS Condition Category

The RIVPACS index calculates the number of reference taxa present in the test sample (observed or "O") and compares it to the number expected to be present ("E") in a reference sample from the same habitat. Calculation of the RIVPACS score is a three-step process. The first step consists of determining the probability of the test sample belonging to four San Francisco Bay Polyhaline reference sample groups. This determination is based on the sampling station's bottom depth and longitude, using a complex linear discriminant function.

The second step is determining, for each sample, the identity and expected number of reference species, based on the probabilities of group membership calculated in Step 1 and the distribution of reference species in each group. In the final step, the number of reference species observed in the sample is counted, the O/E RIVPACS score calculated and compared to the response ranges in Table 4.15 to determine the RIVPACS category and category score.

Table 4.15. RIVPACS category response ranges for San Francisco Bay Polyhaline.

RIVPACS Score	Category	Category Score
> 0.68 to < 1.32	Reference	1
0.33 to 0.68 or 1.32 to 1.67	Low Disturbance	2
0.16 to 0.32 or >1.67	Moderate Disturbance	3
< 0.16	High Disturbance	4

Because of the complexity of the RIVPACS calculations, computer programs are used to determine the O/E values. Detailed instructions for calculating RIVPACS O/E values by two alternate methods are provided in Appendices A and B. Appendix A has instructions for using a web-based tool on the SCCWRP website. Appendix B covers instructions for calculating RIVPACS O/E values using the SAS. The SAS programs calculate RIVPACS O/E values and

condition categories but require availability of the SAS software. The SCCWRP tool is freely available with less rigid data formatting requirements.

Species list contents

The San Francisco Bay Polyhaline species list is provided on a spreadsheet that can be accessed from the SCCWRP website (sccwrp.org) under the Sediment Quality Assessment Tools section of the Sediment Quality research area. The contents of each column on the spreadsheet are described in Table 4.16.

Table 4.16. San Francisco Bay Polyhaline species list contents.

Column	Header	Contents
1	TaxonName	Taxon name
2	Phylum	Taxonomic phylum
3	Class	Taxonomic class
4	Order	Taxonomic order
5	Family	Taxonomic family
6	Mollusc	When present, "Mollusc" indicates molluscan taxa for RBI calculations
7	Crustacean	When present, "Crustacean" indicates crustacean taxa for RBI calculations
8	Amphipod	When present, "Amphipod" indicates amphipod taxa for IBI calculations
9	Tolerance Score	When present, values are tolerance scores for BRI calculation
10	RivColHead	When present, in the abundance data file submitted for RIVPACS calculations to the Utah State University web site, this exact text is used as the column header for abundance data for this taxon
11	RivColNo	When present, in the abundance data file submitted for RIVPACS calculations to the Utah State University web site, this is the column number containing abundances for this taxon.
12	SpeciesLevel	When present, "Drop" in this column indicates that abundances of this taxon are included in index calculations, but it is not included for counting numbers of taxa because lower taxonomic level entries in this taxon are also present.

Integration of Benthic Index Category Scores

The final Benthic LOE category is derived by integrating all four benthic index category scores. The procedure is the same for samples from Southern California Marine Bays and samples from San Francisco Bay Polyhaline. Integration is accomplished by calculating the median of the four individual index category scores. If the median falls between two adjacent categories, the value is rounded up to the next highest integer. The Benthic LOE category names (and corresponding scores) are the same as those described for the individual indices.

Example of Benthic Community Line of Evidence Calculation for Southern California Marine Bays

For the Benthic LOE, the steps involved are gathering the data, calculating benthic community indices, comparing the index values to response ranges, and integrating the individual index results into a single Benthic LOE. While the general process of calculating the indices is similar between habitat types, the details may differ. The following example calculations are for the Southern California Marine Bays habitat. Most of the benthic index calculations can be made with a hand calculator, but it is simpler to use a spreadsheet program, such as Excel.

Data Preparation

A sample data set is shown in Table 4.17. This table presents species abundances for all the benthic organisms found at the station. Each species is designated as sensitive or not, based on a list of sensitive species for the habitat, and identified as to whether it is a mollusc, crustacean, or neither.

Table 4.17. Example benthic community data set.

Species Name	Abundance	Sensitive	Mollusc	Crustacean
Acteocina inculta	296	Yes	Yes	No
Ampithoe valida	9	Yes	No	Yes
Capitella capitata Cmplx	764	No	No	No
Chironomidae	17	No	No	No
Dipolydora sp	73	No	No	No
Exogone lourei	5	Yes	No	No
Geukensia demissa	1	No	Yes	No
Grandidierella japonica	1116	No	No	Yes
Harpacticoida	1	No	No	Yes
Hemigrapsus oregonensis	1	No	No	Yes
Lineidae	1	No	No	No
Marphysa angelensis	9	No	No	No
Marphysa stylobranchiata	2	No	No	No
Mayerella acanthopoda	1	No	No	Yes
Mediomastus sp	2	No	No	No
Monocorophium insidiosum	3	Yes	No	Yes
Musculista senhousia	27	No	Yes	No
Oligochaeta	1584	No	No	No
Podocopida	1	No	No	Yes
Polydora nuchalis	73	No	No	No
Protothaca sp	1	No	Yes	No
Pseudopolydora paucibranchiata	60	No	No	No
Streblospio benedicti	1459	No	No	No
Tagelus subteres	4	Yes	Yes	No
Tryonia sp	2	No	Yes	No
Tubulanus sp	1	No	No	No
Turbellaria	1	No	No	No

Index of Biotic Integrity (IBI)

The specific data needed to calculate the IBI are the total number of taxa, number of mollusc taxa, abundance of *Notomastus* sp., and number of sensitive taxa. The sensitive species list should be from the list specific to the station's habitat.

The IBI metric values for the sample data set are presented in Table 4.18. There were 27 different taxa represented in the sample, 6 of which were molluscs. There were no occurrences of the polychaete, *Notomastus* sp. Finally, there were 5 sensitive species in the sample, which represents 18.5% of the taxa, based on the following:

% sensitive taxa= (number of sensitive taxa/total number of taxa) * 100

Table 4.18. IBI metrics for sample data set.

Metric	Value
Total Number of Taxa	27
Number of Mollusc Taxa	6
Abundance of *Notomastus* sp.	0
Percentage of Sensitive Taxa	18.5

Once the IBI metrics have been calculated, the next step is to compare the values for each of the metrics to a reference range for that specific metric (Table 4.19). The IBI score is set to zero before comparison to the reference ranges. For each metric that is out of the reference range (above or below), the IBI score goes up by one.

For the sample data set, the total number of taxa, number of mollusc taxa and abundance of *Notomastus* sp. all fell within their reference ranges and therefore did not cause the IBI score to rise. However, the percentage of sensitive taxa was below the reference range and therefore caused the IBI score to rise by one. The final IBI score for this data set is thus 1.

Table 4.19. Reference ranges for IBI metrics.

Metric	Reference Range
Total Number of Taxa	13 - 99
Number of Mollusc Taxa	2 - 25
Abundance of *Notomastus* sp.	0 - 59
Percentage of Sensitive Taxa	19 - 47.1

The final step is to compare the IBI score to the category response ranges (Table 4.20) in order to determine the IBI category and score. For the example, the IBI score of 1 corresponds to the Low Disturbance category with a category score of 2.

Table 4.20. IBI category response ranges.

IBI Score	Category	Category Score
0	Reference	1
1	Low Disturbance	2
2	Moderate Disturbance	3
3 or 4	High Disturbance	4

Relative Benthic Index (RBI)

The RBI is the weighted sum of: 1) several community metrics, 2) the abundances of three positive indicator species, and 3) the presence of two negative indicator species.

The first step is to normalize the values for the benthic community metrics relative to the test sample habitat type. In the case of this example the data come from the Southern California Marine Bays habitat. These values are referred to as the scaled values. The calculations use the following four equations:

Total number of taxa/99

Number of mollusc taxa/28

Number of crustacean taxa/29

Abundance of Crustacea/1693

The results of these calculations using the sample data set are shown in Table 4.21.

Table 4.21. Scaled RBI Metric Values.

RBI Metric	Raw	Scaled
Total number of taxa	27	0.272727
Number of Mollusc taxa	6	0.214286
Number of Crustacean taxa	7	0.241379
Abundance of Crustacea	1132	0.668636

The next step is to calculate the TWV. This is calculated using the following:

TWV = Scaled total number of taxa + Scaled number of mollusc taxa + Scaled number of crustacean taxa + (0.25 * Scaled abundance of Crustacea)

For the sample data set the TWV= 0.89555.

Next, the value for the two NIT is calculated. The two negative indicator taxa are *Capitella capitata* complex and Oligochaeta. For each of these taxa that are present, in any abundance whatsoever, the NIT is decreased by 0.1. Therefore, if neither were found the NIT = 0, if both are found the NIT = -0.2. For our example data, both taxa were present, so the NIT = -0.2.

The next step is to calculate the value for the three PIT. The positive indicator taxa are *Monocorophium insidiosum*, *Asthenothaerus diegensis*, and *Goniada littorea.* First, the PIT value is calculated for each species using the following equations:

$$\frac{\sqrt[4]{\textit{Monocorophium insidiosum}\text{ abundance}}}{\sqrt[4]{473}}$$

$$\frac{\sqrt[4]{\textit{Asthenothaerus diegensis}\text{ abundance}}}{\sqrt[4]{27}}$$

$$\frac{\sqrt[4]{\textit{Goniada littorea}\text{ abundance}}}{\sqrt[4]{15}}$$

The three species PIT values are then summed to calculate the PIT value for the sample. If none of the three species is present, then the sample PIT = 0. For the example data, only *M. insidiosum* was present and the result of its calculation was 0.282205, which in the absence of the other species is also the PIT value.

The next step is to calculate the Raw RBI:

Raw RBI = TWV + NIT + (2 * PIT)

For the sample data set:

Raw RBI = 0.89555 + (-0.2) + (2 * 0.282205) = 1.25996

The final calculation is for the RBI Score:

RBI Score = (Raw RBI - 0.03)/4.69

For the sample data set:

RBI Score = (1.25996 - 0.03)/4.69 = 0.26

The last step in the RBI process is to compare the RBI Score to a set of response ranges to determine the RBI category (Table 4.22). For the example, the RBI score falls into the Low Disturbance category, with a category score of 2.

Table 4.22. RBI category response ranges.

RBI Score	Category	Category Score
> 0.27	Reference	1
0.17 to 0.27	Low Disturbance	2
0.09 to 0.16	Moderate Disturbance	3
< 0.09	High Disturbance	4

Benthic Response Index (BRI)

The BRI is the abundance weighted pollution tolerance score of the organisms present in a given benthic community sample. The higher the BRI score, the more degraded the benthic community present in the sample.

The first step in the BRI calculation is to compute the 4th root of the abundance of each taxon in the sample for which pollution tolerance (P) values are available. For the sample data set, the calculated values are found in Table 4.23. The next step is to multiply the 4th root abundance value by the P value, for each taxon (Table 4.23).

Next, separately sum all of the 4th roots of the abundances and all of the products of the 4th roots of abundance and P values (Table 4.23). Any taxa that lack P values are not included in either sum.

The next step is to calculate the BRI score as:

$$\frac{\sum\left(\sqrt[4]{Abundance}\right)\times P}{\sum \sqrt[4]{Abundance}}$$

For the sample data set, the BRI score is 82.56.

The last step is to compare the BRI score to BRI response range values in Table 4.24 to determine the BRI category and category score. For the example, the BRI corresponds to the High Disturbance category, with a category score of 4.

Table 4.23. BRI component calculations for the sample data set. na = pollution tolerance (P) value not available for that taxon.

Taxon Name	Abundance	P	Abundance 4th root	Abundance 4th root * P
Acteocina inculta	296	110.15	4.1478	456.88
Ampithoe valida	9	90.96	1.7321	157.56
Capitella capitata Cmplx	764	130.84	5.2574	687.90
Chironomidae	17	138.87	2.0305	281.99
Dipolydora sp	73	56.56	2.9230	165.33
Exogone lourei	5	41.86	1.4953	62.59
Geukensia demissa	1	na	na	na
Grandidierella japonica	1116	105.98	5.7798	612.57
Harpacticoida	1	32.91	1	32.91
Hemigrapsus oregonensis	1	60.70	1	60.70
Lineidae	1	3.96	1	3.96
Marphysa angelensis	9	97.82	1.7321	169.43
Marphysa stylobranchiata	2	94.27	1.1892	112.10
Mayerella acanthopoda	1	22.26	1	22.26
Mediomastus sp	2	57.84	1.1892	68.78
Monocorophium insidiosum	3	103.42	1.3161	136.11
Musculista senhousia	27	68.05	2.2795	155.12
Oligochaeta	1584	69.96	6.3087	441.35
Podocopida	1	na	na	na
Polydora nuchalis	73	108.42	2.9230	316.91
Protothaca sp	1	55.94	1	55.94
Pseudopolydora paucibranchiata	60	81.68	2.7832	227.34
Streblospio benedicti	1459	61.83	6.1804	382.11
Tagelus subteres	4	37.28	1.4142	52.73
Tryonia sp	2	127.95	1.1892	152.16
Tubulanus sp	1	0.61	1	0.61
Turbellaria	1	44.95	1	44.95
Sum			58.8708	4860.23

Table 4.24. BRI category response ranges and category scores.

BRI Score	Category	Category Score
<39.96	Reference	1
39.96 to 49.14	Low Disturbance	2
49.15 to 73.26	Moderate Disturbance	3
> 73.26	High Disturbance	4

River Invertebrate Prediction and Classification System (RIVPACS)

The RIVPACS index calculates the number of reference taxa present in the test sample (observed or "O") and compares it to the number expected to be present ("E") in a reference sample from

the same habitat. The data needed for the calculation are the latitude, longitude, and depth of the station, along with the taxa names and abundance data (the first two columns from 4.23). For the example data, the station information parameters are: Latitude = 33.64565, Longitude = - 117.88676, with a depth of 5 m and is in the Southern California Marine Bays habitat (habitat C). For SCCWRP online calculator, all of this data is submitting using an Excel template which is provided on the web site.

The computer program calculates the number of expected reference site species (E), which is 4.1794 for the example data set. The number of observed species (O) is also determined, which is equal to five in this case. The RIVPACS score is therefore 1.1964 (5/4.1794).

The score is then compared to the response ranges in Table 4.25 to determine the RIVPACS category and category score. For the example, the RIVPACS score corresponds to the Low Disturbance category, with a category score of 2.

Table 4.25. RIVPACS category response ranges and category scores.

RIVPACS Score	Category	Category Score
> 0.90 to < 1.10	Reference	1
0.75 to 0.90 or 1.10 to 1.25	Low Disturbance	2
0.33 to 0.74 or > 1.25	Moderate Disturbance	3
< 0.33	High Disturbance	4

Integration of Benthic Community Indices

The Benthic LOE category is based on the integration of the four benthic index category scores. The integration is accomplished by calculating the median of the four individual index category scores. If the median falls between two adjacent categories, the value is rounded up. For the sample data set, the index category scores were 2, 2, 2, and 4 for the IBI, RBI, RIVPACS, and BRI, respectively. The median for those values is 2. Therefore, the Benthic LOE for the example is Low Disturbance.

Other California Estuaries and Embayments

For benthic habitats in California that are not classified as C or D – the low salinity portions of San Francisco Bay, the low salinity parts of Southern California Bight, and all other estuaries and embayments throughout California regardless of salinity – we recommend using the modified version of the Multivariate AMBI (M-AMBI) index detailed in Pelletier et al. 2018 with SQO condition thresholds proposed by Gillett et al. 2019. This index is a variation of an

index commonly used by the European Union Water Framework Directive that has been modified, calibrated, and validated for application in all estuaries and embayments of the continental United States.

The M-AMBI is a macrofauna-based condition index that assesses the health and biological integrity for a given location using a combination of pollution sensitive and pollution tolerant taxa (i.e., AMBI score), species richness, species diversity, and oligochaete abundance (Pelletier et al. 2018; Muxika et al. 2007). Over 6,200 estuarine and marine taxa from around the world have been assigned one of five pollution tolerance values, ranging from very sensitive to very tolerant, which are then used to calculate the relative abundance of sensitive and tolerant taxa (following Gillett et al. 2015). Species richness, species diversity, oligochaete abundance, and AMBI scores are integrated together via a factor analysis and compared to modelled expectations of reference and highly degraded sites based upon the location (U.S. West Coast vs. Gulf and East coasts) and bottom water salinity observed at the time of sampling (Table 4.26). Expectations were modelled using benthic infauna, habitat, and contaminant data from the US EPA National Coastal Assessment monitoring program as described in Pelletier et al. (2018). Different habitats were defined by salinity zone (following the Venice Salinity System). Higher salinity habitats were further delineated into West Coast and Non-West Coast (i.e., US Gulf of Mexico and East coasts) because of the notably higher biodiversity of West Coast benthic communities. Note that oligochaete abundance is only used as a metric in tidal freshwater habitats and is used in lieu of species richness.

Table 4.26. Reference and Highly Degraded benchmarks used in the calculation of M-AMBI for each of the different estuarine habitats (following Pelletier et al. 2018).

M-AMBI Habitat	Habitat Condition	AMBI Score Expectation	Species Richness Expectation	Species Diversity Expectation	Oligochaete Abundance Expectation (%)
Tidal Freshwater	Highly Degraded	6		0	100
Tidal Freshwater	Reference	0.15		1.93	0
Oligohaline	Highly Degraded	6	0	0	
Oligohaline	Reference	0.53	16	2.12	
Mesohaline	Highly Degraded	6	0	0	
Mesohaline	Reference	0.85	26	2.48	
Non-West Coast Polyhaline	Highly Degraded	6	0	0	
West Coast Polyhaline	Highly Degraded	6	0	0	
Non-West Coast Polyhaline	Reference	0.72	44	2.96	
West Coast Polyhaline	Reference	0.18	76.8	3.3	
Non-West Coast Euhaline	Highly Degraded	6	0	0	
West Coast Euhaline	Highly Degraded	6	0	0	
Non-West Coast Euhaline	Reference	0.56	61	3.29	
West Coast Euhaline	Reference	0.66	92	3.62	
Hyper Haline	Highly Degraded	6	0	0	
Hyper Haline	Reference	0.32	55	3.45	

The final calculation of M-AMBI scores is a product of a multivariate factor analysis – a complex, iterative statistical method that would be difficult to precisely calculate by hand. As such, it is recommended that factor analyses are conducted with a standardized statistical computer program (e.g., R, Matlab, SAS). Even then, the calculation of M-AMBI scores can be non-trivial, so we have prepared a function within the R statistical computing language to calculate M-AMBI scores and assign condition categories for SQO assessments, as well as for more general benthic condition assessment studies. Suggested M-AMBI score thresholds for assigning SQO condition categories were developed using data from San Francisco Bay (Table 4.27).

Table 4.27. M-AMBI condition category thresholds for SQO application (following Gillett et al. 2019).

M-AMBI Score	Condition Category	Category Score
≥ 0.578	Reference	1
0.483 - < 0.578	Low Disturbance	2
> 0.387 - < 0.483	Moderate Disturbance	3
≤ 0.387	High Disturbance	4

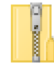
Using the M-AMBI Calculator

The M-AMBI calculator and associated files are embedded as a zipped file in this document or can be obtained from the Sediment Quality Assessment Tools section at www.sccwrp.org. In order to run the M-AMBI R script, one will need to have base R (the analytical underpinnings) and R Studio (a good program to interface with R) installed on a computer. All the development and testing of this work has been done on a PC running Windows.

R can be downloaded for free from: https://cran.r-project.org/

R Studio can be downloaded for free from: https://www.rstudio.com/

Once you have the R and R Studio programs installed, you can start unpacking the calculator files and working with the M-AMBI script. First unzip the downloaded file by double clicking on the folder icon below and save the unzipped folder and its contents onto your local machine.



MAMBI Calculator 11-22-19.zip

When unzipped, the contents should look like Figure 4.2.

Name	Date modified	Type	Size
.Rproj.user	11/13/2019 12:06 ...	File folder	
.Rhistory	10/25/2019 3:30 PM	RHISTORY File	40 KB
EQR.R	9/25/2018 1:32 AM	R File	1 KB
Final MAMBI Tool.Rproj	2/17/2020 11:58 AM	R Project	1 KB
Gillett et al AMBI EG schemes EI appendix...	2/13/2019 5:57 PM	Microsoft Excel C...	207 KB
MAMBI calculator-djg - alt config.R	11/22/2019 5:40 PM	R File	14 KB
MAMBI calculator-djg.R	11/22/2019 5:49 PM	R File	14 KB
Pelletier2018_Standards.xlsx	11/14/2019 12:49 ...	Microsoft Excel W...	10 KB
Ref - EG Values 2018.csv	10/1/2018 11:14 PM	Microsoft Excel C...	260 KB

Figure 4.2. An image of unzipped folder containing the R scripts and associated files.

From this file folder, double-click the “Final MAMBI Tool.Rproj” file; this is an R Studio Project that will open R Studio and associate contents of the folder in the R Studio environment. In the lower right pane of R Studio (the file pane) double click on the “MAMBI calculator-djg.R” link; this will open the script into the Source pane (upper left), which should look like Figure 4.3. Instructions for application of the M-AMBI function, the input file format, and the data needed to calculate M-AMBI are detailed at the top of the script (text in upper left pane of Figure 4.3). The calculator will not work if the format is not followed exactly. The input file must be an xlsx file and it should look like Table 4.28, including all of the named fields.

Table 4.28. An example of the data input file format for the M-AMBI calculation function. All fields must be included with this spelling and capitalization.

StationID	Replicate	SampleDate	Latitude	Longitude	Species	Abundance	Salinity
A1	1	6/3/2019	33.69402	-118.037036	*Leitoscoloplos pugettensis*	22	28
A1	1	6/3/2019	33.69402	-118.037036	*Streblospio sp*	57	28
A1	1	6/3/2019	33.69402	-118.037036	*Americorophrium*	2	28
Z2092	1	7/6/2019	33.749729	-118.117294	*NoOrgansimsPresent*	0	32

Field names:

StationID - an alpha-numeric identifier of the location
Replicate - a numeric identifying the replicate number of samples taken at the location
SampleDate - the date of sample collection
Latitude - latitude in decimal degrees
Longitude - longitude in decimal degrees must include a negative sign for the Western coordinates)
Species - name of the fauna, ideally in SCAMIT ed12 format, do not use sp. or spp., use sp only or just the genus - if no animals were present in the sample use NoOrganismsPresent with 0 abundance
Abundance - the number of each Species observed in a sample
Salinity - the salinity observed at the location in PSU, ideally at time of sampling

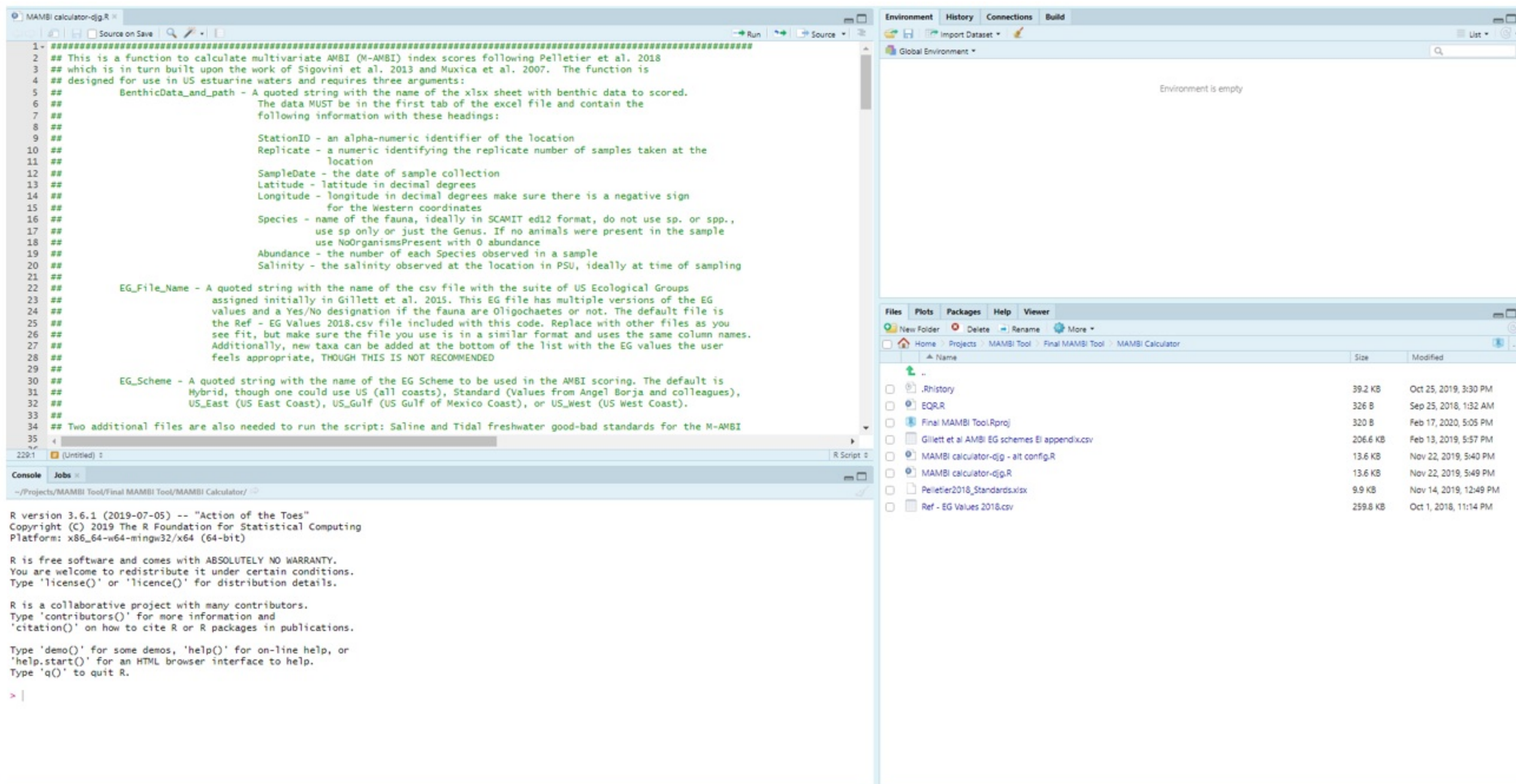


Figure 4.3. An image of R Studio with MAMBI Calculator loaded into Source pane (upper left).

As noted in the instructions, the following R packages must be installed on your machine for the MAMBI function to work: 1. tidyverse; 2. reshape2; 3. vegan; and 4. readxl. Packages can be installed via the tools tab at the top or directly using the function “install.packages()”. Once installed, highlight all of the text in the Source window and run it. This will enable the MAMBI.DJG function into your R Studio session. Upon success, it should be listed in the Functions portion of the Environment pane (upper right) of R Studio (Figure 4.4).

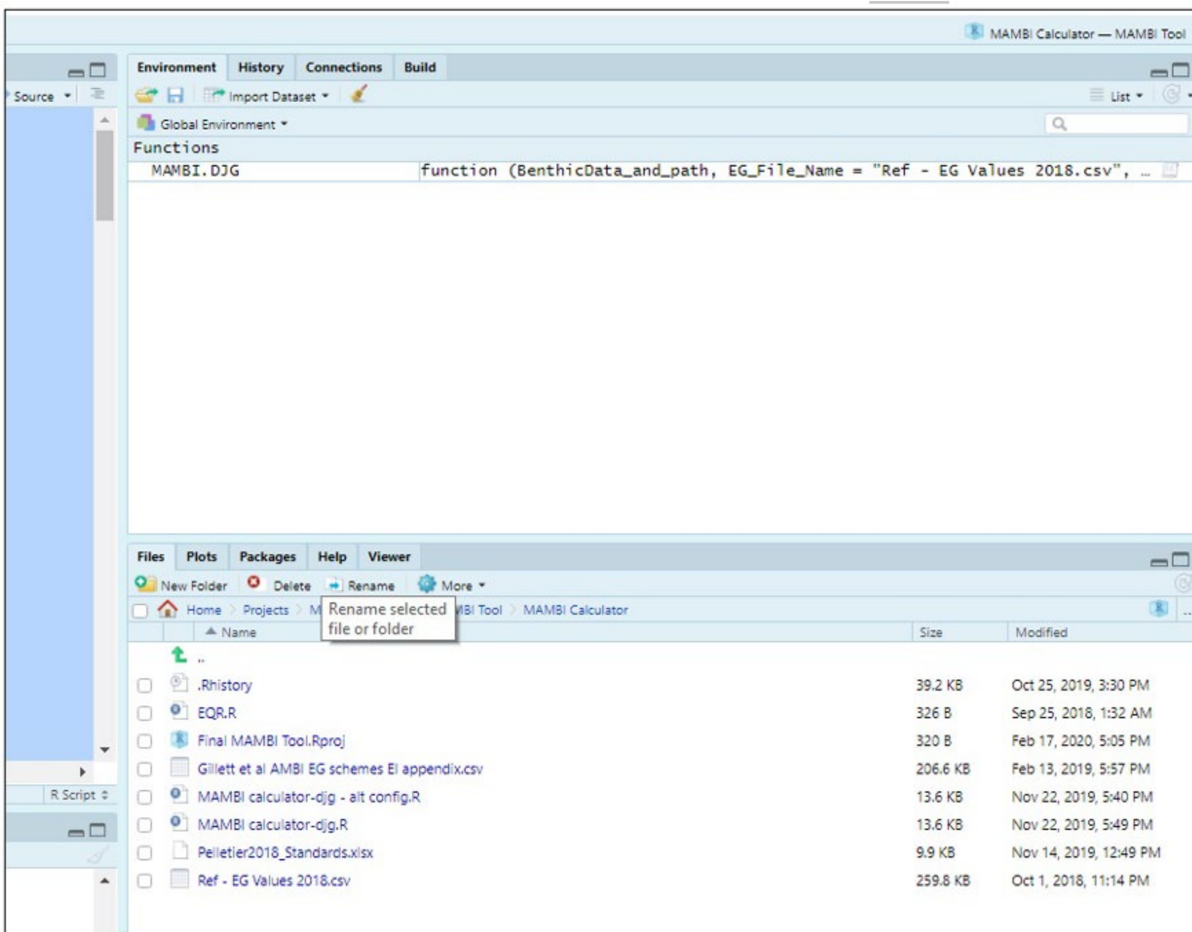


Figure 4.4. An image of R Studio Environment pane once the MAMBI.DJG function has been enabled.

Once the function has been enabled in your R Studio session, open a new script in R Studio. This is where you can start using the function to calculate M-AMBI. As noted in the instructions, the MAMBI.DJG function has three arguments. All three arguments must be present to calculate MAMBI scores.

1. BenthicData_and_path – This is the path and xlsx file name of the benthic data you want to analyze contained in quotation marks. **This must be specified by the user.** Note that you have to change the slashes in a normal windows path from back slash (\) to forward slashes (/) for R. Also note that if the data are not exactly in the format specified in the instructions, the function will not work.

2. EG_File_Name – This is the name of the csv file that contains the tolerance values the M-AMBI uses. **Default value is "Ref – EG Values 2018.csv" and is a file included in the zipped folder.** If the user wants to supply their own tolerance values, we suggest editing this file or provide a path and file name for a new file of their own. However, we recommend simply using the standard tolerance values unless the user is an experienced benthic ecologist.
3. EG_Scheme – This is the column name within the csv file containing the tolerance values. **The default value is "Hybrid"**. The hybrid scheme has the best set of tolerance values identified during the calibration and validation of this M-AMBI tool. If the user wants to use a different EG scheme, they can choose among the options in the csv file or supply their own (though this not recommended for novice users).

The first step in using the MAMBI.DJG function is to name a dataframe into which the function will put its results. Begin by opening a new, blank R script. Within that script name an object (test.df, in this illustration) and assign the output of the MAMBI.DJG function to it. In this example, the default settings are used and therefore we only need to specify a quoted string with the name and associated path of the benthic data. The Source pane (upper left) should look like Figure 4.5, albeit with your own pathway to the benthic data. Note that R uses forward slashes (/) in its directory designations, while Windows uses backslashes (\). As such they will have to be changed if copying and pasting directly from Windows.

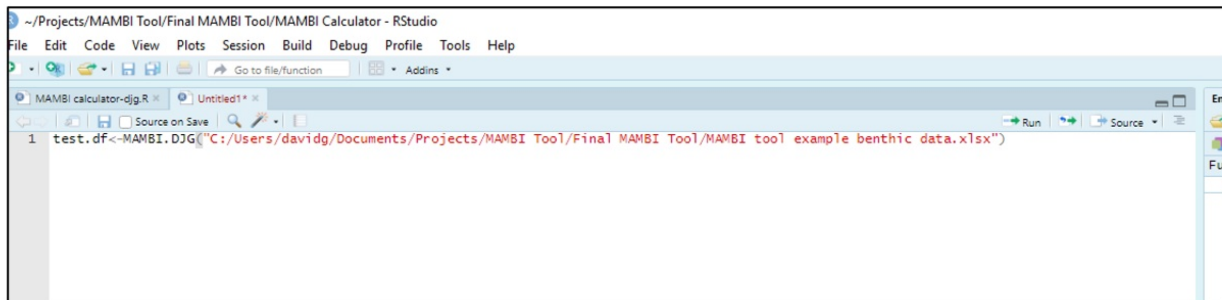


Figure 4.5. An image of the R Studio Source pane with the code to calculate M-AMBI scores for the example data set.

Highlight this line of code and run it. This will produce a data frame called test.df that contains the results of the M-AMBI scoring function. This test.df can be opened in R or exported for viewing in another program using a variety of different functions (e.g., write.table(), write.csv()). Exporting the data frame is the best way to save the results of the calculation. As noted in the MAMBI.DJG instructions, the output file will contain the following fields:

StationID, Replicate, and SampleDate – Which combine to represent a unique sample.

Latitude and Longitude – Location of the sample.

SalZone – The salinity zone the function assigned the sample, based upon its observed salinity, and used to set index expectations of reference and highly disturbed (WPH= Western Polyhaline, MH = Mesohaline, OH = Oligohaline, TF = Tidal Freshwater).

AMBI_Score, S, and H – Component metrics used to calculate M-AMBI.

MAMBI_Score – The index score for that sample.

Orig_MAMBI_Condition – Condition category the M-AMBI score corresponding to the scheme of Pelletier et al. (2018).

New_MAMBI_Condition – Condition category the M-AMBI score corresponding to SQO Benthic LOE categories (Table 4.27).

Use_MAMBI – Yes/No qualifier indicating if the M-AMBI was appropriate to apply to the sample.

Use_AMBI – Yes/No/Cautiously qualifier indicating the confidence one should have in the M-AMBI score. It is based upon the % of the abundance in a sample that was assigned a tolerance value and following recommendations of Borja and Muxika (2005).

YesEG – The percent of the abundance in a sample that had a tolerance value assigned to it.

The values in the MAMBI_Score field are converted to SQO condition scores (New_MAMBI_Condition) as detailed in Table 4.27 and then can be used as the Benthic LOE for SQO assessment for any of the non-C or D benthic habitats in California.

Example of Benthic Community Line of Evidence Calculation for Low Salinity Estuaries

Producing the Benthic LOE for samples from the low salinity (< 27 PSU) portions of San Francisco Bay and Southern California, as well as all other estuaries and embayments of any salinity across California, entails gathering the data, calculating M-AMBI index score, and comparing the index value to response ranges. We recommend using the R-based M-AMBI calculator (MAMBI.DJG) in R Studio to obtain index scores.

Data Preparation

A sample data set is shown in Table 4.29. This table presents species abundances for all the benthic organisms found at the station, as well as station information on date of collection, location, and salinity. These data must be saved in a Microsoft Excel xlsx file.

Table 4.29. Example data (benthic fauna and station information) to calculate SQO Benthic LOE for a mesohaline estuary location.

StationID	Replicate	SampleDate	Latitude	Longitude	Species	Abundance	Salinity
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Tubificidae	143	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Ampelisca abdita	23	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Corophium heteroceratum	6	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Sphaerosyllis californiensis	4	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Theora lubrica	4	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Polydora cornuta	3	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Glycinde picta	2	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Harmothoe imbricata Cmplx	2	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Cirratulidae	1	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Glycinde sp SF1	1	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Grandidierella japonica	1	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Heteromastus sp	1	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Monocorophium acherusicum	1	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Musculista senhousia	1	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Pseudopolydora kempi	1	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Spionidae	1	17.8
RMP-BB15	1	2/21/1996	37.616667	-122.2833	Venerupis philippinarum	1	17.8

Calculating M-AMBI scores

The data file is submitted to the MAMBI.DJG function. The output from this function will be a dataframe that should be converted by the user to a csv file and saved to the user's computer. Note that the function does not automatically save the file; the user has to actively do it themselves.

Interpreting M-AMBI Scores

The output of the M-AMBI calculator tool (Table 4.30) indicates that the sample had a score (MAMBI Score) of 0.511, which corresponds to an SQO condition category (New MAMBI Condition field) of Low Disturbance, corresponding to an SQO Condition Category Score of 2. As only one benthic index is used in this analysis, the sample would also have a Benthic LOE score of 2, which would then be integrated with the corresponding toxicity and sediment chemistry LOE scores for this sample to determine the overall assessment condition.

Table 4.30. M-AMBI calculator output from the example data. M-AMBI Score and New M-AMBI Condition are used for the SQO Benthic LOE.

StationID	Replicate	SampleDate	Latitude	Longitude	SalZone	AMBI Score	S	H	Oligo pct
RMP-BB15	1	2/21/1996	37.61667	-122.2833	MH	5.07	17	1.65	NA

StationID	MAMBI Score	Orig MAMBI Condition	New MAMBI Condition	Use MAMBI	Use AMBI	YesEG
RMP-BB15	0.511	Moderate	Low Disturbance	Yes	Yes	98.5

Chapter 5: Sediment Toxicity

Sediment toxicity provides two types of information in this assessment: 1) the potential bioavailability of contaminants and 2) a measure of contaminant biological effects. Multiple toxicity tests are needed to assess toxicity because no single method exists that can capture the full spectrum of potential contaminant effects. Toxicity assessment under the California Sediment Quality Objectives (CASQO) framework requires information from two types of tests: 1) short-term amphipod survival and 2) a sublethal test.

Objectives

This chapter provides a description of the sediment toxicity test methods specified under the SQO policy. This document is intended to supplement published toxicity protocols by providing information on specific aspects of the methods that are used in many California monitoring programs so that future analyses will yield comparable and high-quality results. This chapter also provides instructions for interpreting toxicity data relative to the CASQO assessment framework.

Scope

The sediment toxicity methods described in this manual are based on an evaluation of methods conducted by Greenstein *et al.* (2008). These toxicity methods include both standardized tests of amphipod survival and sublethal tests using polychaete (*Neanthes arenaceodentata*) growth in sediment and mussel (*Mytilus galloprovincialis*) embryo development at the sediment-water interface (SWI). Many other types of sediment toxicity tests are used to assess sediment quality (e.g., pore water and elutriate tests), but they are not included in this manual because they have not been specified for use in the CASQO program. While the CASQO program is limited to application in bays and estuaries, the toxicity methods described here are also appropriate for assessing sediment toxicity in other habitats (e.g., offshore waters), as long as the exposure conditions are within the tolerance range of the species.

General Study Considerations

Selection of Test Species

The various species used in toxicity tests often have different tolerances to sediment physical characteristics (e.g., grain size), sensitivities to contaminants, and associations between response and sediment exposure. There is no single “perfect” toxicity test method or species that can measure all aspects of sediment toxicity that are important for sediment quality assessment. Consequently, a suite of multiple toxicity methods is needed to provide a complete assessment of sediment toxicity. At a minimum, this suite should include at least one short-term survival test and one sublethal test.

The sediment toxicity methods described in this chapter are summarized in Table 5.1. A variety of other methods are used in other programs to assess toxicity, but only those specified in the CASQO program are described here. The SQO policy requires the use of at least one acute test using amphipods and one sublethal test from Table 5.1. Use of alternate methods may be valuable as a supplement to those in Table 5.1, but they are not used in the determination of the Toxicity line of evidence (LOE).

Table 5.1. Sediment toxicity test methods recommended for use in California marine habitats.

Test Type / Species	Taxonomic Group	Matrix	Duration (days)	Endpoint(s)
Acute				
Eohaustorius estuarius	Amphipod	Whole Sediment	10	Survival
Leptocheirus plumulosus	Amphipod	Whole Sediment	10	Survival
Rhepoxynius abronius	Amphipod	Whole Sediment	10	Survival
Sublethal				
Neanthes arenaceodentata	Polychaete	Whole sediment	28	Growth, Survival
Mytilus galloprovincialis	Mussel	Sediment-water Interface	2	Embryo Development

The tolerance of the test species to the characteristics of the test sample should be considered when selecting the test methods for an individual study. For example, extremes in sediment particle size may influence the survival response of an amphipod, which may confound interpretation of the results. Potential confounding factors include, but are not limited to, grain size, total organic carbon (TOC) content, ammonia, and salinity. Where known, information regarding sensitivity to confounding factors is presented in tables within the description of each method.

Another important factor to consider when choosing test methods is the suspected toxicants of concern at a location. For example, *Eohaustorius estuarius* has a relatively high tolerance to copper and may not be a sensitive measure of sediment toxicity where copper is the primary toxicant (McPherson and Chapman 2000). Therefore, it would be a poor choice for testing under these conditions. Conversely, amphipods are very sensitive to organophosphorus and pyrethroid pesticides.

Finally, choice of test method may be dictated by project-specific objectives or conditions. If historical data are available from a site, it may be best to use the same test species that was previously used to make temporal comparisons for trends analyses. For regional monitoring programs, use of a consistent suite of tests is helpful in increasing data comparability among surveys or regions. The test method selection may also be helpful in investigating the results of prior studies. For example, if benthic community data indicate that a particular taxonomic group of organisms is impacted at a site, then using a member of that taxon in a toxicity test may clarify the cause of the impact.

Sample Preparation

Chapter 1 provides information on field collection methods and sample storage. Unlike chemistry and benthic infauna samples, toxicity samples cannot be stored for extended periods. The toxicity tests should be started within one month of sample collection (2 weeks recommended) to minimize potential changes in toxicity due to storage.

Sediment for all methods except the SWI test should be press sieved to remove native animals that might be either predators or be the same species as a test organism. Press sieving consists of forcing the sediment through a 2-mm mesh screen without adding water beyond that which was

already associated with the sample naturally. Press sieving is not necessary for the SWI test because the test organisms are enclosed within a screened chamber that prevents the entry of predators.

The sediment sample should be homogenized in the laboratory prior to addition to the test chambers. Regardless of whether the sample was originally homogenized at the time of collection, it should be homogenized in the laboratory to ensure that each replicate test chamber contains a representative sample. Sediment cores used for the SWI test are not homogenized. As a result, the SWI test results may show greater variability between replicates as a result of small-scale variation in sediment characteristics. This increased variation is a consequence of the test design and is not an indication of poor technique. The test response values used to interpret the results take this variation into account.

Animal Acclimation

All the test species in Table 5.1 are available from commercial vendors who either collect them from the field or raise them in culture facilities. Availability of test animals from commercial sources is never guaranteed and should be confirmed in the planning stages of a study. The test animals used in each method must be acclimated (i.e., with respect to temperature and salinity) to test conditions within each laboratory prior to the start of testing. The acclimation period required for each species is variable. The duration for each species can be found in a table within each method description section. The amphipod *Leptocheirus plumulosus* and the polychaete *N. arenaceodentata* can be cultured and laboratories may choose to use animals from in-house cultures rather than from commercial sources.

Interpretation of Test Results

Interpretation of the test results for use within the CASQO assessment framework requires the test response to be classified into one of four categories:

- Nontoxic: Response not substantially different from that expected in sediments that are uncontaminated and have optimum characteristics for the test species (*e.g.*, control sediments).
- Low Toxicity: A response that is of relatively low magnitude; the response may not be greater than test variability.
- Moderate Toxicity: High confidence that a statistically significant toxic effect is present.
- High Toxicity: High confidence that a toxic effect is present, and the magnitude of response includes the strongest effects observed for the test.

The test response category is determined by comparing the results to a set of response ranges that are specific to the test species (Table 5.2). Classification of the test response requires four types of summary data for each test: 1) mean control response, 2) mean response for test sample, 3) test sample response expressed as a percentage of the control, and 4) determination of statistical significance of result from control. Once the toxicity test data are properly formatted, results for each individual toxicity test are simply compared to the ranges for the response categories shown in Table 5.2. Additional instructions for analyzing the test response data are presented as flow

charts in the method descriptions for each test species and in the example at the end of this chapter.

Table 5.2. Sediment toxicity response classification ranges.

Test Species/Endpoint	Nontoxic (Percent)	Low Toxicity (Percent of Control)	Moderate Toxicity (Percent of Control)	High Toxicity (Percent of Control)
Eohaustorius Survival	90 -- 100	82 -- 89[a]	59 -- 81[b]	< 59
Leptocheirus Survival	90 -- 100	78 -- 89[a]	56 -- 77[b]	< 56
Rhepoxynius Survival	90 -- 100	83 -- 89[a]	70 -- 82[b]	< 70
Neanthes Growth	90 -- 100[c]	68 -- 89[a]	46 -- 67[b]	< 46
Mytilus Normal Development	80 -- 100	77 -- 79[a]	42 -- 76[b]	< 42

[a] If the response is not significantly different from the negative control, then the response is classified as Nontoxic.
[b] If the response is not significantly different from the negative control, then the response is classified as Low Toxicity.
[c] Expressed as percentage of control.

The toxicity response classification ranges were established for each test organism by analyses of data for California samples using the methods described in Greenstein *et al.* (2008). The ranges are based on the following criteria:

- The range representing the Nontoxic category is equivalent to the control acceptability criterion for the test method.
- The lower bound of the Low Toxicity category range is based on the 90th percentile Minimum Significant Difference (MSD) that is specific to each test species.
- The lower bound of the Moderate Toxicity category range is based on the mean of two values:
  - the 99th percentile MSD value
  - the test response corresponding to the 75th percentile of toxic samples.

Acute Test Methods

Amphipod 10-day Survival

All three acute methods use species of amphipods in 10-day whole sediment exposures. Two of these species, *Eohaustorius estuarius* and *Rhepoxynius abronius,* have been used in numerous monitoring and assessment studies in California (Fairey *et al.* 1998; Bay *et al.* 2000; Bay *et al.* 2005). Tests using *Leptocheirus plumulosus* have been used infrequently in California, but have been widely used in monitoring and assessment studies on the East and Gulf coasts (McGee *et al.* 1999; Lewis *et al.* 2006) and are required for testing drilling muds (Federal Register 2001). All three species are burrowers: *E. estuarius* and *R. abronius* burrow freely, and *L. plumulosus* lives in U-shaped burrows.

E. estuarius and *R. abronius* are collected from the field by commercial vendors for use in toxicity testing. As such, conditions of temperature and salinity are variable at the collection

sites. *L. plumulosus* can either be cultured in the laboratory or collected in the field but has the same temperature and salinity requirements for acclimation, whether coming from culture or field conditions. It is important that the animals be brought slowly to test conditions before acclimation begins. Temperature must not be adjusted more than 3°C per day and salinity not more than 5 g/kg per day.

Test Method

The methodology for all three methods can be found in USEPA (1994) and ASTM (1996). Test parameters for each method can be found in Table 5.3. Each method is conducted in 1-L chambers containing 2 cm of test sediment and approximately 800 ml of overlying water. As shown in Table 5.3, standard test salinity and temperature vary between methods, but for all methods, samples should be gently aerated, and tests should be conducted under constant light. At least five replicates of each treatment must be tested.

To prepare for tests, the test chambers should be set up with sediment, water, and light aeration the day before animals are added. Twenty amphipods in the 2- to 5-mm range are then added to each replicate chamber. Animals caught in the surface tension in the exposure beakers should be sunk by gently dropping water on them. Food is not provided during testing for any of the methods, and there is no renewal of overlying water during the exposure period.

On day 0 and day 10 of the test water-quality measurements including temperature, dissolved oxygen (DO), pH, salinity, and ammonia are recorded in the overlying water in a surrogate replicate test chamber for the control and each test sediment. Subsampling porewater for analysis of salinity and ammonia is also recommended prior to test initiation.

Daily observations are performed on each replicate to ensure proper aeration and to document any unusual animal behavior or obvious abnormalities (e.g., bacterial growth on the surface of the sediment).

On day 10 of the test sediments are sieved through a 0.5-mm screen to capture and count surviving amphipods.

Table 5.3. Test characteristics for 10-day acute amphipod exposures using *Eohaustorius estuarius*, *Leptocheirus plumulosus*, and *Rhepoxynius abronius*.

Parameter	*E. estuarius*	*L. plumulosus*	*R. abronius*
1. Temperature	15 ±1°C	25 ±1°C	15 ±1°C
2. Salinity	20 ±2 g/kg	20 ±2 g/kg	28 ±2 g/kg
3. Illuminance	500 - 1000 lux	500 - 1000 lux	500 - 1000 lux
4. Photoperiod	Continuous light	Continuous light	Continuous light
5. Acclimation	2 - 10 days at test temperature and salinity	2 - 10 days at test temperature and salinity	2 - 10 days at test temperature and salinity
6. Size and life stage	3 - 5 mm	2 - 4 mm no mature animals	3 - 5 mm
7. Number of organisms/chamber	20	20	20
8. Number of replicates/treatment	5	5	5
9. Aeration	Enough to maintain 90% saturation	Enough to maintain 90% saturation	Enough to maintain 90% saturation
10. Water quality measurements	Temperature daily. pH, salinity, ammonia and DO of overlying water at T_0 and T_{final}. Pore water pH, salinity, ammonia at T_0 and T_{final}.	Temperature daily. pH, salinity, ammonia and DO of overlying water at T_0 and T_{final}. Pore water pH, salinity, ammonia at T_0 and T_{final}.	Temperature daily. pH, salinity, ammonia and DO of overlying water at T_0 and T_{final}. Pore water pH, salinity, ammonia at T_0 and T_{final}.
11. Feeding	None	None	None
12. Test acceptability criteria	Mean control survival of ≥ 90 and ≥ 80% survival in each replicate.	Mean control survival of ≥ 90 and ≥ 80% survival in each replicate.	Mean control survival of ≥ 90 and ≥ 80% survival in each replicate.
13. Grain size tolerance	0.6 - 100% sand	0 - 100% sand	10 - 100% sand
14. Ammonia tolerance	< 60 (total, mg/L)	< 60 (total, mg/L)	< 30 (total, mg/L)
15. Total sulfide tolerance	1.9 (mg/L)	Not Available	1.5 (mg/L)

Quality Assurance

A 10-day, water-only reference toxicant test using ammonia should be performed simultaneously with each set of field samples tested. Most previous protocols have used 4-day tests with cadmium. Use of cadmium as a reference toxicant is also acceptable; however, ammonia is preferable because 1) it can be measured easily in the laboratory, 2) it is a confounding factor often associated with contaminated sediments, and 3) it does not present the safety concerns and disposal issues associated with cadmium. Whichever reference toxicant is chosen, each laboratory must establish a control chart consisting of at least three tests and no more than the 20 most recent tests.

The median Effective Concentration (EC50) is the concentration of a toxicant that induces a response (i.e., percent mortality) that is halfway between the baseline and maximum possible

effect. The EC50 for un-ionized ammonia or cadmium for each test performed should fall within two standard deviations of the mean of the previous tests on the control chart. A test falling outside two standard deviations should trigger a review of all data and test procedures to assure the data are of good quality.

All test batches must include a negative control. The negative control should consist of sediment from the amphipod collection site or sediment as free of known contamination as possible and having previously been demonstrated to meet test control acceptability requirements. If using *R. abronius* with stations having a grain size content greater than 90% fines (silt + clay), a grain size control with a range of particle sizes like the test sediments should be included. A grain size control may also be useful for interpreting tests using *E. estuarius* that are conducted in sediments with a high fines content.

Each of the amphipod species has a specific tolerance to ammonia, as measured in the test chamber overlying water (Table 5.3). If any of the chambers within a test exceed this ammonia concentration, 50% of the overlying water in all chambers within the experiment may be changed up to twice per day until all are below the target concentration. The mean control survival for each test batch must be 90% or greater and each control replicate, individually, must have at least 80% survival. In addition, water quality parameters must be within acceptable limits and initial size ranges for the amphipods must be followed.

Data Analysis and Interpretation

The final response category for the test result is based on two parameters: whether the response is significantly different from the negative control and the magnitude of the response. Statistical comparisons between negative controls and test samples are conducted using a one-tailed Student's t-test assuming unequal variance (Zar 1999).

For purposes of interpretation and comparison to response ranges, the data from test samples must be control normalized as follows:

(mean survival of test sample / mean survival of control) * 100

Note that the response ranges for the Nontoxic category are based on non-normalized percent survival (except for *Neanthes* growth), but that normalized values are used for comparison to the Low, Moderate, and High response ranges. Values should be rounded to the nearest whole percentage.

After statistical analysis and control normalization, the data are compared to response ranges to determine the response category for each sample. The ranges are specific to each test species and are provided in Table 5.2. The test result interpretation process is also illustrated in the form of a flow chart for each amphipod species (Figures 5.1 through 5.3).

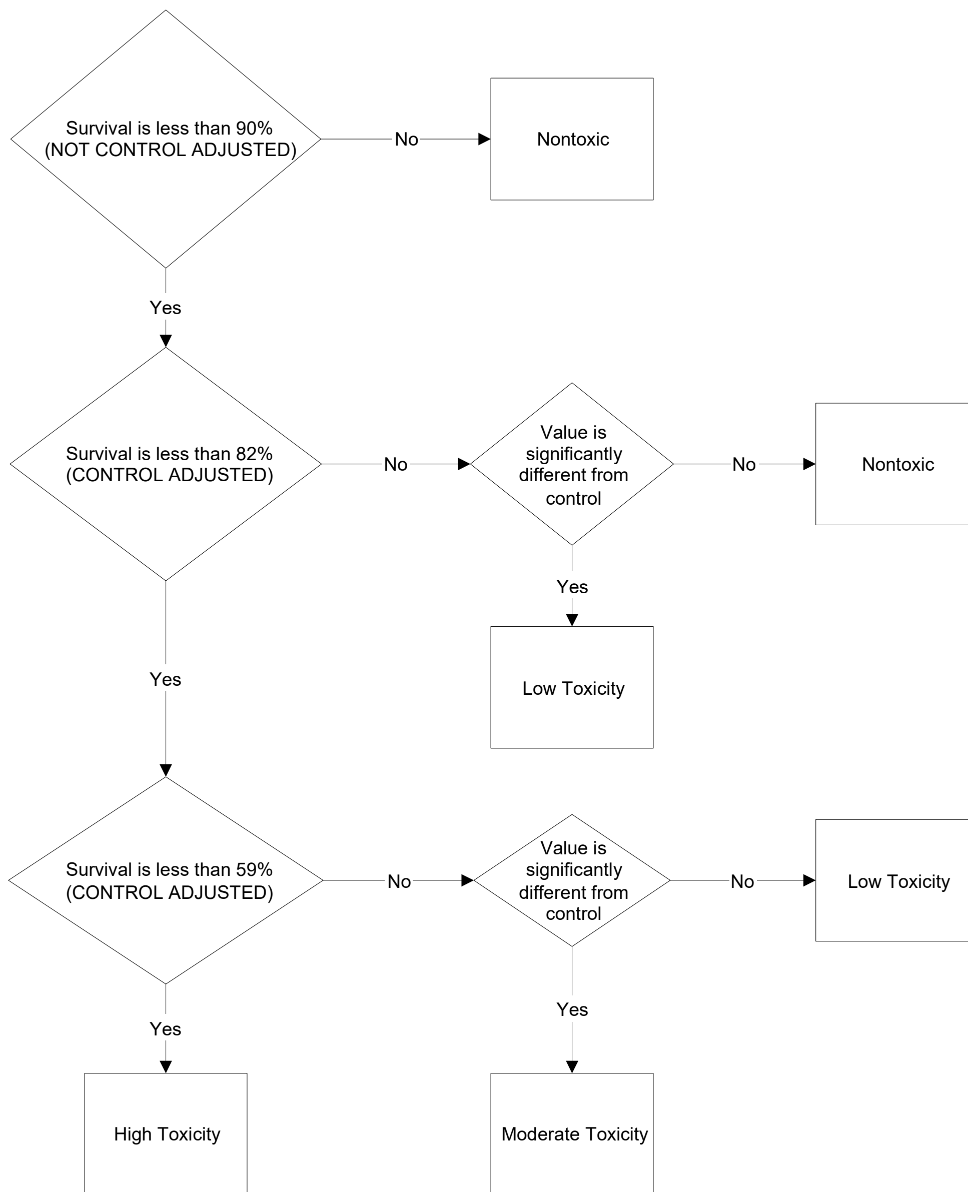


Figure 5.1. Flow chart for determining the *E. estuarius* toxicity response category.

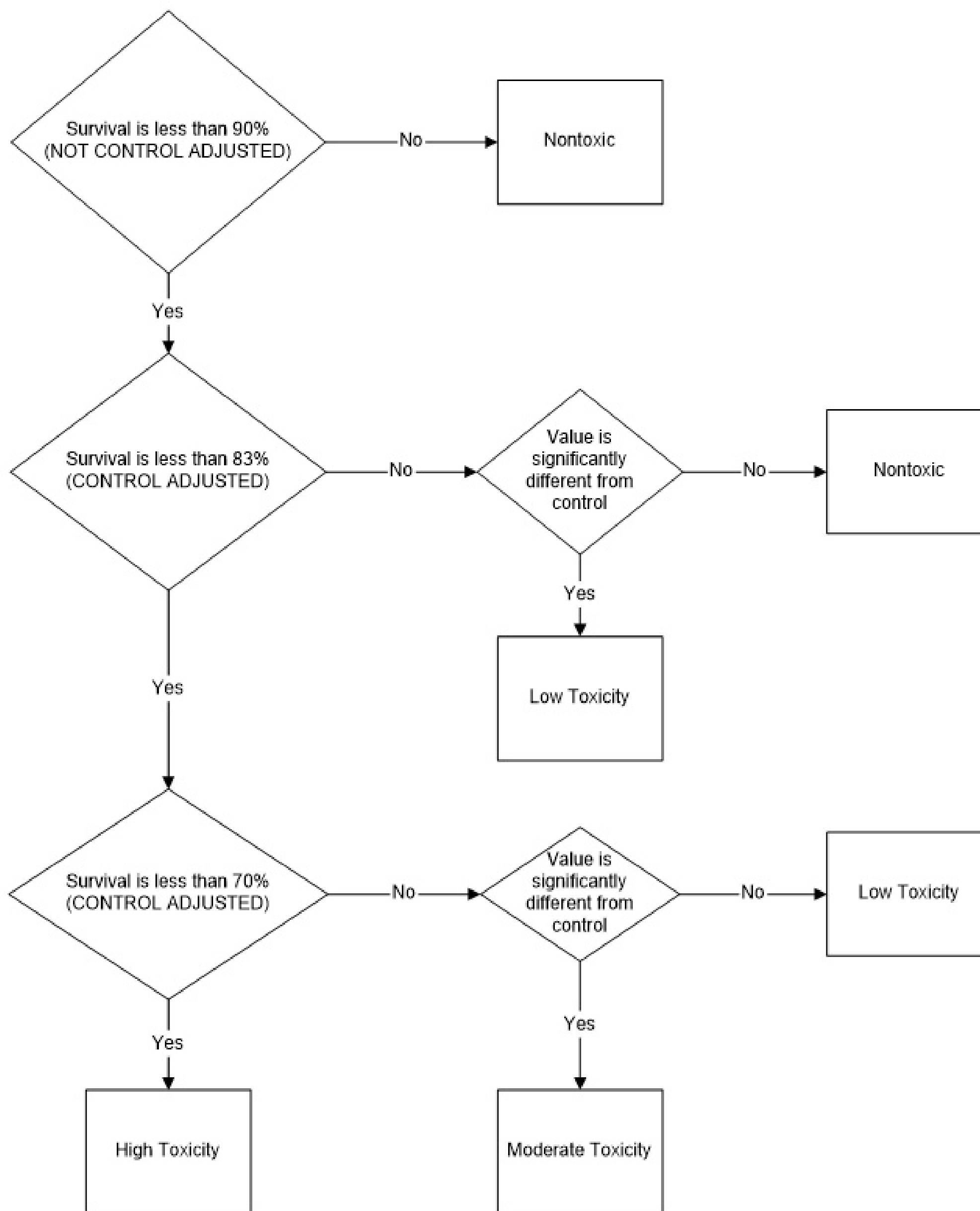


Figure 5.2. Flow chart for determining the *R. abronius* toxicity response category.

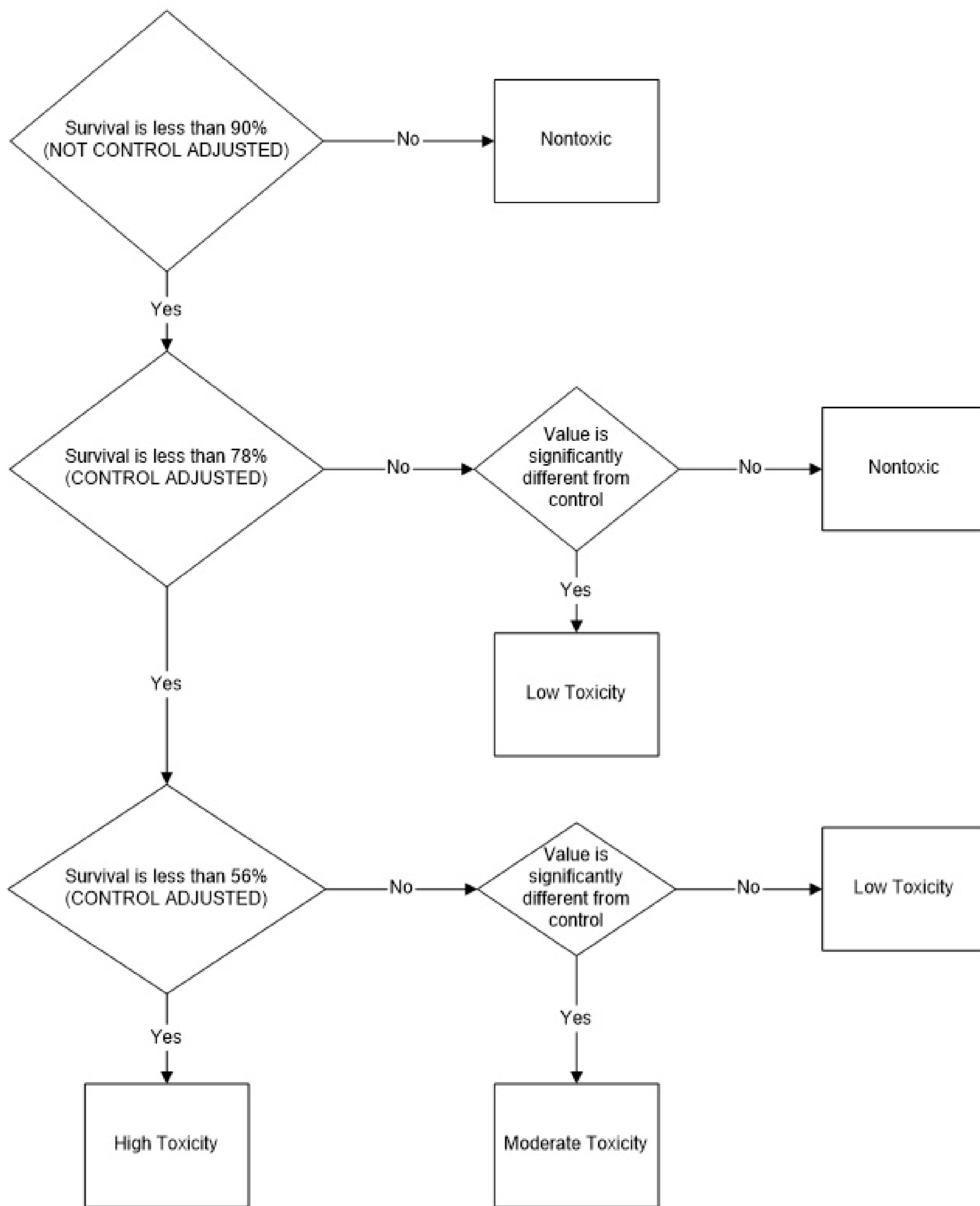


Figure 5.3. Flow chart for determining *L. plumulosus* toxicity response category.

Neanthes arenaceodentata 28-day Growth and Survival

N. arenaceodentata is widely distributed throughout the world in sandy or muddy sand sediments (Reish 1985). The animals live in non-permanent mucoid tubes and are deposit feeders on sediment particles (Bridges and Farrar 1997). *Neanthes* can be cultured in the laboratory and are raised commercially for use in toxicity tests. Instructions for maintaining a laboratory culture can be found in ASTM (2002).

N. arenaceodentata has been used for sediment toxicity testing for about 40 years (Reish 1985). Testing methods include both 10-day survival tests and longer-term exposures with growth and/or reproduction components. A 20-day version of the growth test has been used in the state of Washington for many years (PSWQA 1995). A 28-day exposure method has been developed with modifications to make the test more sensitive and reliable (Bridges and Farrar 1997; Bridges *et al.* 1997; Gardiner and Niewolny 1998; Lotufo *et al.* 2000). The recommended 28-day method is described below**.**

The 28-day *Neanthes* method recommended for the CASQO program is a revision of guidance published by ASTM (2002). Details of the method are described in Farrar and Bridges (2011). The following method description is based on these two publications. The major modifications from the ASTM version are:

- Utilization of ≤ seven-day-old, post-emergent juveniles instead of two- to three-week-old worms. Initiating with younger juveniles was found to increase the sensitivity of the test (Bridges and Farrar 1997).
- Reduction of the exposure chamber volume from 1 L to 300 ml. This change increased the manageability of the test by decreasing both sediment and overlying water volume requirements.
- Reduction of the number of worms per chamber from five to one. Fewer animals per replicate decreased intra-chamber variability in organism size and reduced the overall number of worms needed to conduct a test.
- Increase in the number of replicates per treatment from 5 to 10. Greater replication increased the statistical power of the test.

Test Method

The day prior to starting an experiment, approximately 75 ml of homogenized sediment should be added to 12 replicate 300 ml tall-form beakers to obtain the required depth of 2 cm. Note that two of the beakers will serve as surrogates used for sediment pore water ammonia measurements and therefore will not have animals added to them. The sediment is then overlain with 125 ml of 30 g/kg seawater. Either natural 0.45-µm filtered seawater or artificial seawater may be used. Beakers are then gently aerated and maintained at 20°C and with a light cycle of 12:12 hours light:dark. A listing of all parameters for the *Neanthes* test can be found in Table 5.4.

Table 5.4. Test characteristics for 28-day *Neanthes arenaceodentata* growth and survival test.

Parameter	*N. arenaceodentata*
1. Temperature	20 ±1°C
2. Salinity	30 ±2 g/kg
3. Illuminance	500 - 1000 lux
4. Photoperiod	12:12 hours light:dark
5. Acclimation	1 day at test temperature and salinity
6. Size and life stage	≤7 days post-emergent juveniles
7. Number of organisms/chamber	1
8. Number of replicates/treatment	10
9. Aeration	Enough to maintain 90% saturation
10. Water quality measurements	Temperature daily. pH, salinity, ammonia and DO of overlying water at T_0 and T_{final}. Prior to each water change pH, salinity, ammonia and DO of overlying water in 3 replicates per treatment. Pore water pH, salinity, ammonia at T_0 and T_{final} from surrogate beakers.
11. Feeding	Twice per week. 2 mg of Tetramarin® on one day and 2 mg of Tetramarin® plus 2 mg of alfalfa on the other.
12. Test acceptability criteria	Mean control survival of 80% and positive growth in controls.
13. Grain size tolerance	5 - 100% sand
14. Ammonia tolerance	<20 (total, mg/L)
15. Total sulfide tolerance	<5 (mg/L)

On day 0, *N. arenaceodentata* (≤ seven days post-emergence) are placed into counting chambers (one animal per chamber): one chamber for each exposure beaker plus an additional five for initial weight measurement. Counting chambers are randomly assigned to each exposure beaker and the initial weight group. The contents of each counting chamber are then gently transferred to their corresponding beaker. Animals caught in the surface tension in the exposure beakers should be sunk by gently dropping water on them.

The five animals for initial weight measurement should be rinsed in de-ionized water, placed on tared pans, and dried in an oven at 60°C for 24 hours. After 24 hours in the drying oven, the pans are removed, allowed to cool in a desiccator, and then weighed to obtain initial weight for growth calculations.

Starting on day 0 of the test run, water-quality measurements (including dissolved oxygen, pH, salinity, and ammonia) are taken from the overlying water in each test chamber. These measurements should be taken in three replicates per sediment. The same measurements are repeated at least once weekly thereafter, always prior to water changes (see below). Some laboratories measure DO daily during the test. Exposure temperature (min/max) is also monitored and recorded daily. Observations of each replicate beaker are conducted daily and should include whether worms are on the surface of the sediment and how, if at all, sediment appearance has changed. In addition, pore water ammonia must be determined at day 0 and at the end of the exposure in the two surrogate beakers with no worms.

Water must be exchanged (~60 ml) from each beaker once per week after water quality parameters are measured. The worms are fed twice per week, separated by about three days (e.g., Tuesdays and Fridays). Each beaker is provided with 2 mg of Tetramarin® (Tetra Sales, Blacksburg, Virginia) one day and 2 mg of Tetramarin® plus 2 mg of alfalfa on the other. Both the Tetramarin® and the alfalfa are ground to 0.5 mm and are delivered to the exposure containers in a seawater slurry.

On day 28, the sediment contained in each beaker is gently sieved (using a 425-µm-mesh sieve) and surviving worms are recovered. Surviving worms are counted and recorded. Survival is determined by gently prodding animals with a blunt probe. If movement is observed, the animal is considered to be alive. Worms that are unaccounted for are considered to be dead. Surviving animals in each replicate should then be rinsed with de-ionized water, put on pre-weighed pans and placed in a drying oven at 60°C for 24 hours. After 24 hours in the drying oven, the pans are removed, allowed to cool in a desiccator then weighed to obtain the individual dry weight for each replicate/animal to the nearest 0.1 mg.

Quality Assurance

A 4-day, water-only reference toxicant test using ammonia should be performed simultaneously with each set of field samples tested. Each laboratory must establish a control chart consisting of at least three tests and tracking no more than the 20 most recent. The EC50 for each test performed should fall within two standard deviations of the mean of the previous tests on the control chart. A test falling outside two standard deviations should trigger a review of all data and test procedures to assure that the data are of good quality.

All test batches must include a negative control. The negative control should consist of sediment as free of known contamination as possible and having previously been shown to meet test control acceptability requirements.

Total ammonia concentrations above 20 mg/L have been found to have a negative impact on both survival and growth (Dillon *et al.* 1993). If pore water ammonia concentration, as measured in the surrogate beakers, in any sediment treatment (station) is greater than 20 mg/L, then all chambers should undergo up to twice daily 50% water changes until all treatments fall below this level. Addition of animals cannot occur until acceptable ammonia concentrations are present.

The mean control survival for each test batch must be 80% or greater, and there must be measurable positive growth in the controls. In addition, water quality parameters should be within acceptable limits.

Data Analysis and Interpretation

Growth rate is calculated using the equation:

$$G = \frac{DWTt_2 - DWTt_1}{T}$$

Where: $DWTt_2$ = the mean dry weight (mg) of surviving animals in a treatment at test termination;

$DWTt_1$ = the mean dry weight of the initial group of animals; and

T = the duration of the test in days.

The growth rate is therefore expressed in units of mg/day.

Statistical comparisons for the growth endpoint are achieved using a one-tailed Student's t-test assuming unequal variance (Zar 1999). For purposes of interpretation and comparison to response ranges, the data from test samples must be control normalized for growth as follows:

(mean growth of test sample / mean growth of control) * 100

After statistical analysis and control normalization, the data are compared to the ranges in Table 4.2 to determine the response category for each sample. Note that the *Neanthes* test is the only method in this document where control normalized data is used to determine whether the response is classified as Nontoxic. Figure 5.4 illustrates the data interpretation process in the form of a flow chart.

Survival is calculated in each treatment group by dividing the number of surviving animals by the number of animals at the start. Statistical comparisons between negative controls and test samples for the survival endpoint must use categorical statistics since there are only two possible outcomes per replicate, dead or alive. Fisher's exact test is the method used for the survival endpoint (Zar 1999). The survival endpoint is not used for the toxicity response classification but can be used as ancillary data in assessment of data quality and sediment condition.

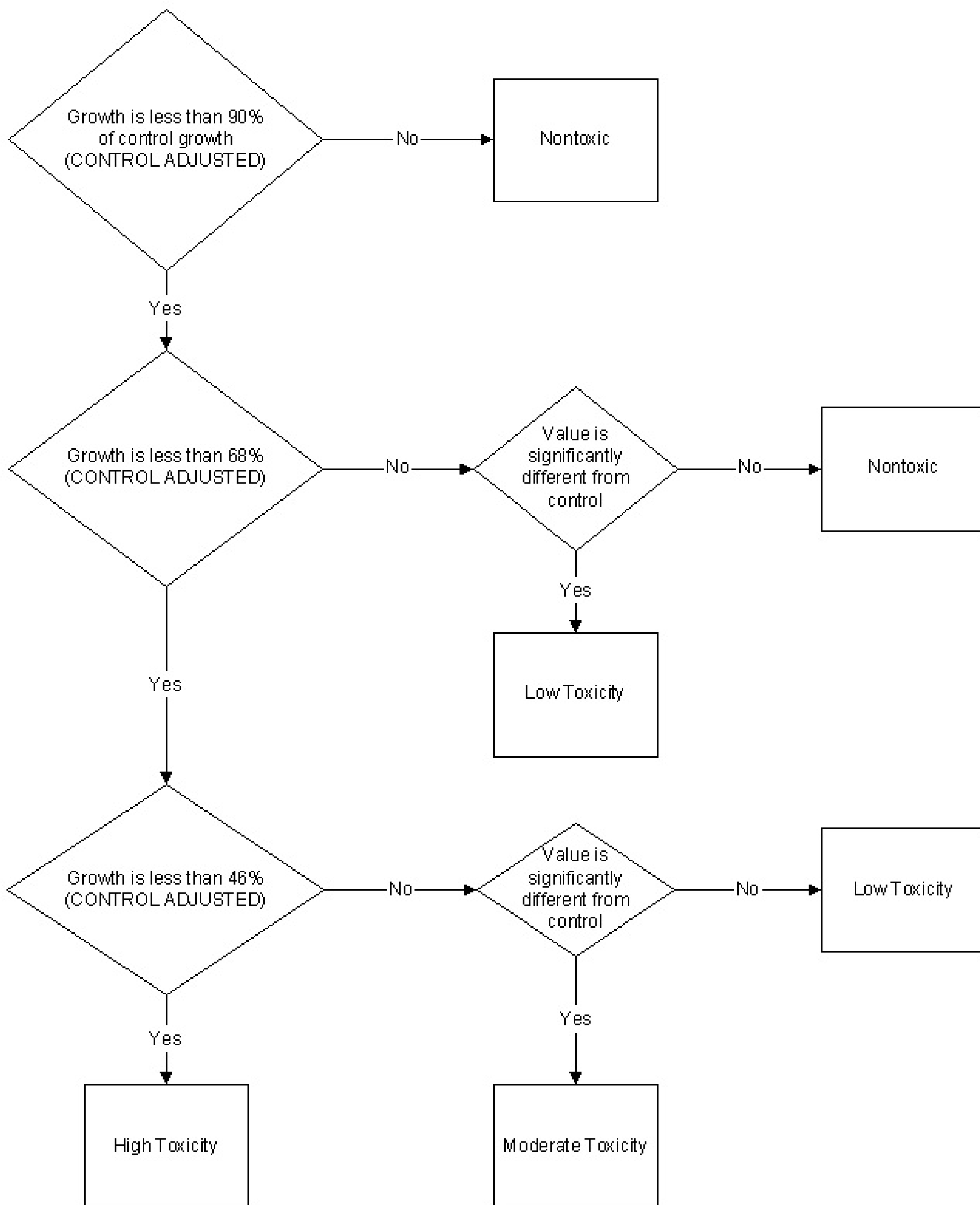


Figure 5.4. Flow chart for determining *N. arenaceodentata* toxicity response category.

Mytilus galloprovincialis 2-day Embryo Test at the Sediment-Water Interface

Use of embryos from the mussel *M. galloprovincialis* for toxicity testing is common in California and is part of the USEPA West Coast methods (USEPA 1995). However, most of these tests have been performed on aqueous samples, as opposed to sediment. The method described here is a modification of the exposure apparatus to allow for testing sediment from intact core samples at the interface between the sediment and overlying water, or SWI. This method has been used in a regional monitoring program in San Francisco Bay (SFEI 2001).

Contaminants in the sediment can be an important source of toxicity to the water column (Burgess *et al.* 1993). This flux of contaminants out of the sediment would be expected to have its greatest effect on toxicity where the sediment and overlying water meet. Therefore, the method described here measures an important component of sediment toxicity that is usually not investigated.

Details of the exposure system can be found in Anderson *et al.* (1996) and methods for the preparation and handling of the mussel embryos are in USEPA (1995). A listing of all test parameters can be found in Table 5.5.

Table 5.5. Characteristics for 2-day mussel (*Mytilus galloprovincialis*) embryo development test at the sediment-water interface.

Parameter	*Mytilus galloprovincialis*
1. Temperature	15 ±1°C
2. Salinity	32 ±2 g/kg
3. Illuminance	500 - 1000 lux
4. Photoperiod	16:8 hours light:dark
5. Acclimation	2 days at test temperature and salinity; up to 4 weeks
6. Size and life stage	Newly fertilized eggs
7. Number of organisms/chamber	~ 250
8. Number of replicates/treatment	4
9. Aeration	Enough to maintain 90% saturation
10. Water quality measurements	Temperature daily; pH, salinity, ammonia and DO of overlying water at T_0 and T_{final} from surrogate core tube
11. Feeding	None
12. Test acceptability criteria	Mean control percent normal-alive of ≥ 80%; meet all water quality limits
13. Grain size tolerance	0 - 100% sand
14. Ammonia tolerance	< 4 (total, mg/L)
15. Total sulfide tolerance	< 0.09 (mg/L)

Test Method

Sediment is generally collected in polycarbonate core tubes (7.5 cm diameter) with polyethylene caps. A 5-cm depth of sediment is collected. There must be at least 8 cm between the top of the sediment and the top of the core tube in order to allow room for the screen tube that will hold the embryos for the test. A minimum of four cores should be collected for toxicity testing from each station. At least one additional core should be collected for water quality measurements. Intact cores should be transported with overlying water from the sediment collection in place. Approximately 24 hours prior to test initiation, all but about 0.5 cm of the overlying water should be siphoned off and gently replaced with 300 ml of clean seawater. The core tubes are then placed at 15°C with gentle aeration.

Field collection of sediment cores (e.g., from a grab sample) is preferred, because this provides the most undisturbed sample for testing. However, homogenized sediment samples may also be used. If the latter approach is taken, the homogenized sediment should be loaded into the test chambers in the laboratory, as described below, to simulate the core sample. The maximum holding time for homogenized sediment used in the SWI test is 4 weeks. However, it is highly recommended that SWI tests be initiated as soon as possible, or within 14 days of sediment sampling, to minimize change in sediment characteristics.

If homogenized sediments are to be tested, the sediments should be press sieved with a 2-mm stainless steel sifting screen. After homogenization and sieving, 5 cm of sediment is added to the same type of core tube used for field collection. Then 300 ml of 32 ppt, 15°C seawater should be added. Approximately 2 cm of free space should be left at the surface to accommodate displacement due to eventual inclusion of the aerator and screen tube in the test chamber. Sediment should be allowed to settle and equilibrate 24 hours before initiation of test.

On the day of test initiation, polycarbonate screen tubes with 37-µm mesh are gently added to each core tube (Figure 5.5). Details of screen tube construction can be found in Anderson *et al.* (1996). When the tubes are placed on the sediment, the bottom collar should rest so that the screen is elevated approximately 1 cm above the substrate. The water level outside the tube should be approximately 0.5 cm below the top of the screen tube. If necessary, water may need to be siphoned from the outside of the screen tube to achieve the proper level. Once the test chamber has been set up, there should be about 150 ml of water inside the screen tube. At this point, the aeration should be directed inside the screen tube.

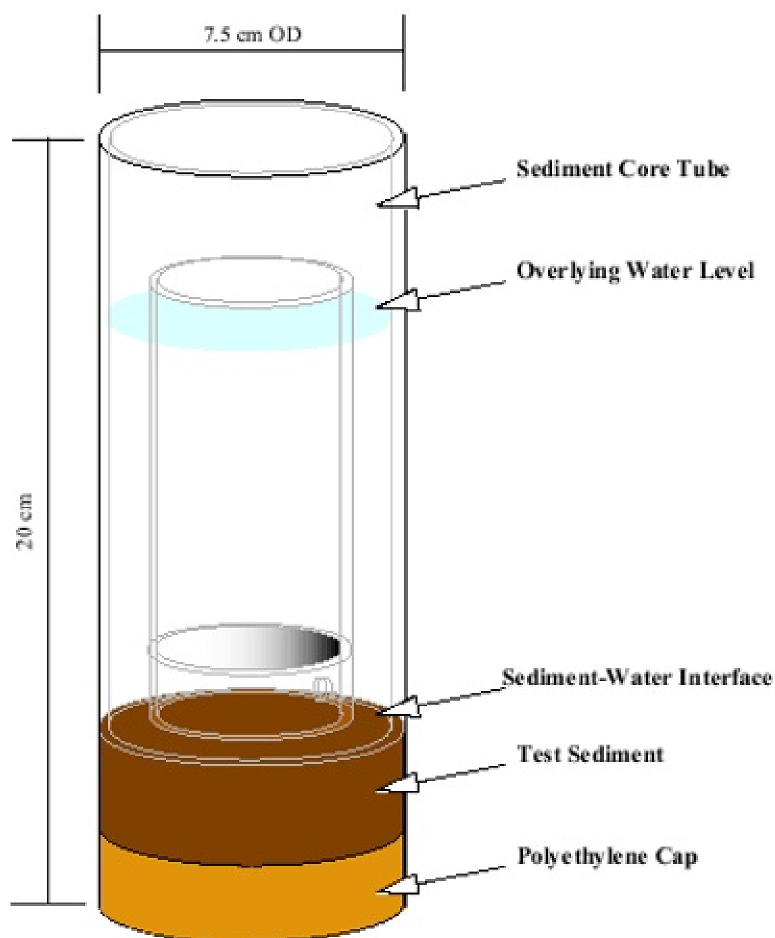


Figure 5.5. Schematic diagram of sediment-water interface exposure system (Anderson *et al.* 1996).

Mussel brood stock can be obtained from commercial vendors or collected from the field in areas known to be free of contaminants. Adult mussels should be acclimated to laboratory conditions for at least two days prior to testing and may be held in the laboratory for up to four weeks. However, ocean water temperature variations often make it difficult to hold the animals without spawning. Frequently, mussels are successfully used on the day of collection or day of arrival at the lab when shipped.

Fertilized mussel eggs are prepared as described in the EPA manual (USEPA 1995). Approximately 250 embryos are introduced to the screen tube of each replicate. It is important to add the same number of embryos to each replicate. An additional 5 scintillation or shell vials with 10 ml of seawater must be prepared, and the same volume of embryo stock added to these containers. These additional samples are used to determine the initial quantity of embryos. The initial samples should be preserved immediately and counted using a microscope.

Water quality parameters (dissolved oxygen, pH, salinity and ammonia) should be made prior to test initiation on day 0 and at test termination. Temperature should be monitored continuously.

Daily observations should be made on each replicate with special attention to aeration, sediment condition (e.g., anoxia, microbial growth such as a bacterial/diatom mat) and the presence of any invertebrates in the sediment cores.

At the end of the exposure period, the screen tubes are removed from the sediment and the embryos are washed into glass scintillation or shell vials with seawater squirt bottles. Care must be taken to recover all embryos from the screen. Preservative is then added to the vials and the embryos are examined microscopically to determine if they are normally developed. An inverted microscope is recommended. This allows for viewing the embryos through the bottom of the vial and is thus faster than using a Rafter cell. This approach has the additional advantage of not exposing technicians to preservative fumes. When evaluating the embryos with the microscope, all embryos present in each vial must be observed and scored as normally or abnormally developed. Normally developed embryos have a distinctive “D” shape (Figure 5.6). Embryos not possessing this shape are scored as abnormal. Embryos that appear normal but do not contain internal tissues are also counted as normal, but it is recommended that they be enumerated separately.

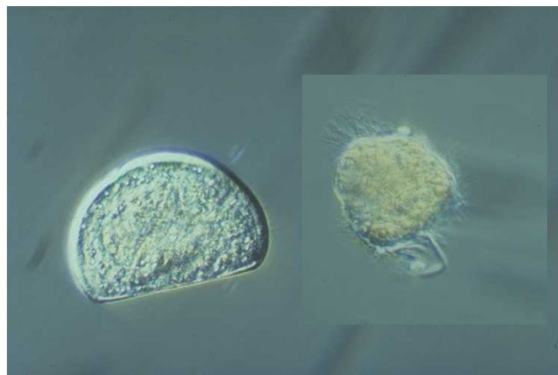


Figure 5.6. Normal (left) and abnormal (right) *M. galloprovincialis* embryos after 48 hours of development.

Quality Assurance

A 2-day, water-only reference toxicant test using ammonia or copper should be performed simultaneously with each set of field samples tested. Each laboratory must establish a control chart consisting of at least three tests and no more than the 20 most recent. The EC50 for each test performed should fall within two standard deviations of the mean of the previous tests on the control chart. A test falling outside two standard deviations should trigger a review of all data and test procedures to assure that the data are of good quality.

M. galloprovincialis embryos are quite sensitive to ammonia. The water overlying the sediment in the core tube should not have a total ammonia concentration exceeding 4 mg/L at the start of an exposure. If any station within an experiment exceeds this level, then the overlying water in all exposure chambers in the test batch should be replaced until none exceed this level.

All test batches must include both chamber and seawater negative controls. The chamber negative control should consist of seawater in a core tube with no sediment and a screen tube placed inside. This control tests for any toxicity associated with the exposure system. The seawater negative control usually consists of seawater inside a scintillation or shell vial. This control verifies the health of the organisms. The control from the simultaneous reference toxicant exposure may serve for the seawater negative control.

Care must be taken that the correct species is being used. It has been found that there are differences in sensitivity between *M. galloprovincialis* and the other commonly used species *Mytilus edulis* (Bryn Phillips, personal communication). Organisms should be identified to species by competent personnel using morphological characteristics and appropriate keys. Animals purchased from culture facilities are assumed to be the correct species.

The mean control percent normal-alive for each test batch must be 80% or greater. In addition, water quality parameters must be within acceptable limits.

Data Analysis and Interpretation

The method endpoint in this document is different from the USEPA manual (USEPA 1995). The endpoint determined for this method is calculated as follows:

(# of normal embryos/initial # added) * 100

The results are expressed as percent normal-alive (PNA). This endpoint takes into account the difficulty in finding abnormal embryos microscopically among the sediment grains that are usually carried into the vial when the embryos are rinsed from the screen tube. The assumption is also made that missing and abnormal embryos are not alive at the end of the exposure. Note that by counting the abnormal embryos as well, the traditional percent normal can also be calculated.

Statistical comparisons between negative controls and test samples are achieved using a one-tailed Student's t-test assuming unequal variance (Zar 1999). For purposes of interpretation and comparison to established response ranges, the data from test samples must be control normalized:

(mean PNA of test sample/mean PNA of control) * 100

After statistical analysis and control normalization, the data are compared to response ranges to determine the toxicity response category for each sample (Table 5.2; Figure 5.7). Note that non-normalized values are used for determining the Nontoxic category, and normalized values are used for comparisons to the other category ranges.

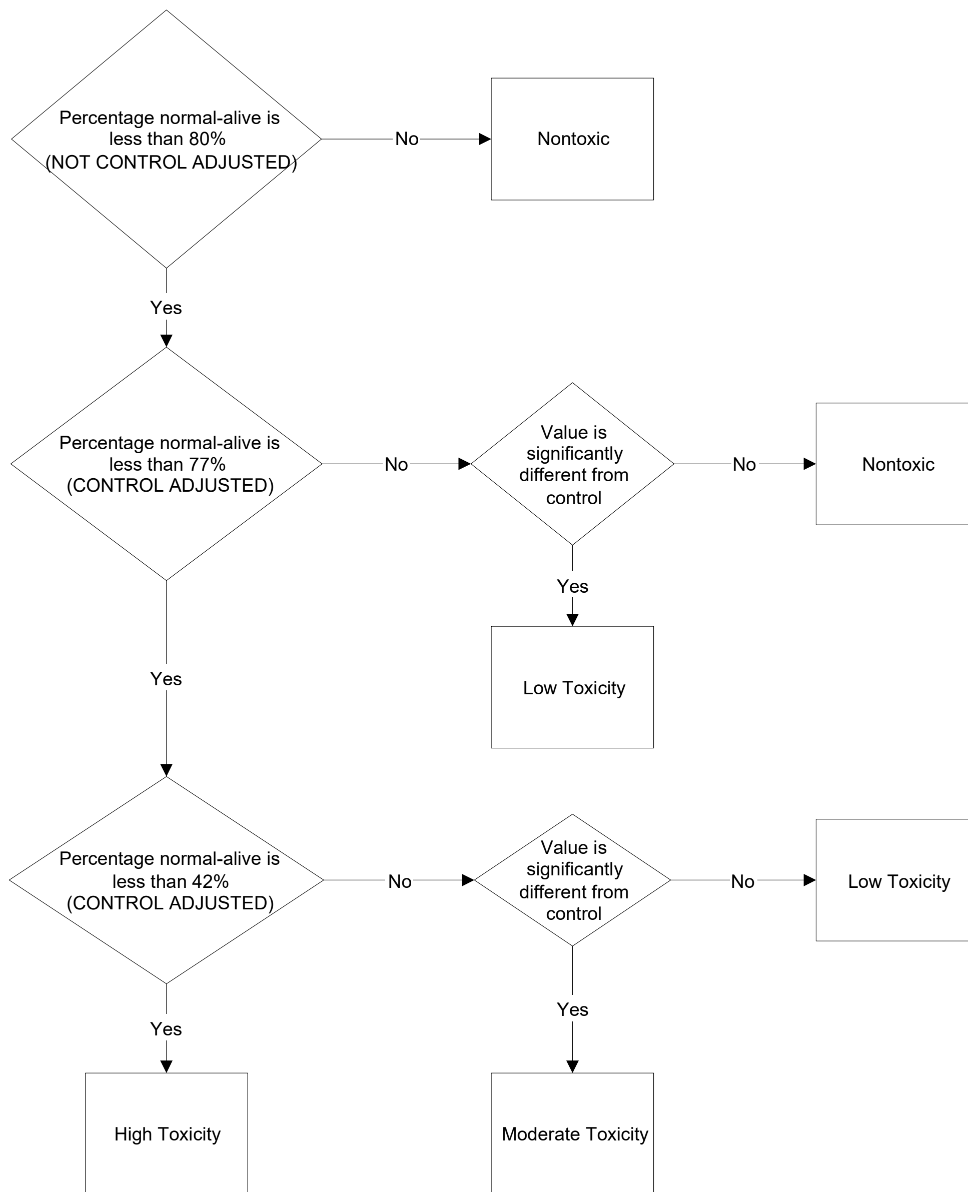


Figure 5.7. Flow chart for determining *Mytilus* embryo development response category using the SWI test.

Integration of Toxicity Test Results

To determine the Toxicity LOE category for a given station, the results of the individual toxicity tests must first be transformed into numeric values. Numeric category scores are assigned to each toxicity test result as follows: Nontoxic = 1, Low Toxicity = 2, Moderate Toxicity = 3, High Toxicity = 4. The scores of all tests are then averaged to yield the station's Toxicity LOE category, with each test result weighted equally in the calculation.

If calculated means have decimal values of 0.5 or higher, they are rounded up to the nearest category. If means have decimal values of less than 0.5, they are rounded down. The scheme relating numeric scores with category names is the same for the Toxicity LOE as for the individual tests (e.g., Moderate Toxicity = 3).

Data Management

Data should be collected and formatted in such a manner that it can be incorporated into a regional sediment quality database. Examples of database formats can be obtained from the Surface Water Ambient Monitoring Program (SWAMP), the Southern California Bight Regional Monitoring Program, and Regional Monitoring Program for Water Quality in the San Francisco Estuary (RMP). The SWAMP data format for toxicity can be found at https://www.waterboards.ca.gov/water_issues/programs/swamp/swamp_iq/toxicity.html. Information on the RMP database format is available through www.sfei.org. The Bight database can be found on the SCCWRP website (www.sccwrp.org).

The electronic data records must include the following:

- Station and sample collection information
- Toxicity raw and summarized data
- Statistical results
- Water quality data collected during toxicity testing

In addition, it is important to keep records regarding any anomalies that occur during testing (e.g., power failure or why a replicate was missing). These records may help with data interpretation and should be included in comments fields within the database.

Example of Toxicity Line of Evidence Calculation

Data Preparation

The raw data from at least two toxicity test methods are compiled and the mean response (e.g., % survival) for each sample is calculated. The response data must be control normalized ((data from assessment station/control data) * 100). T-tests must be performed on the raw data from the assessment station versus control response. A sample data set containing results from two tests, the amphipod *E. estuarius* survival test and sediment-water interface test using the mussel *M. galloprovincialis* embryo development, is shown in Table 5.6.

Table 5.6. Toxicity data used in the example.

Test Method	*E. estuarius* survival	*M. galloprovincialis* Percent Normal Alive
Raw Station Response	90%	57%
Raw Control Response	92%	92%
Control Normalized Response	98%	62%
Statistical Difference from Control	No	Yes

Individual Toxicity Test Result Classification

The data from each toxicity test are compared to a series of response ranges that are unique to each test method (Table 5.2). Note that in the case of *Eohaustorius* and *Mytilus*, for the Nontoxic category, the *non-normalized* mean response for the assessment station is compared to the range, whereas for the Moderate and High toxicity categories, the *control-normalized* response is compared to the ranges. Figures 5.8 and 5.9 show the data classification results for each test organism. The toxicity category is based on both the response level and whether a statistically significant difference is present. The raw *Eohaustorius* survival value of 90% classifies it in the Nontoxic category (Figure 5.8) and the *Mytilus* percent normal-alive value of 62% (control normalized) classifies is in the Moderate Toxicity category (Figure 5.9).

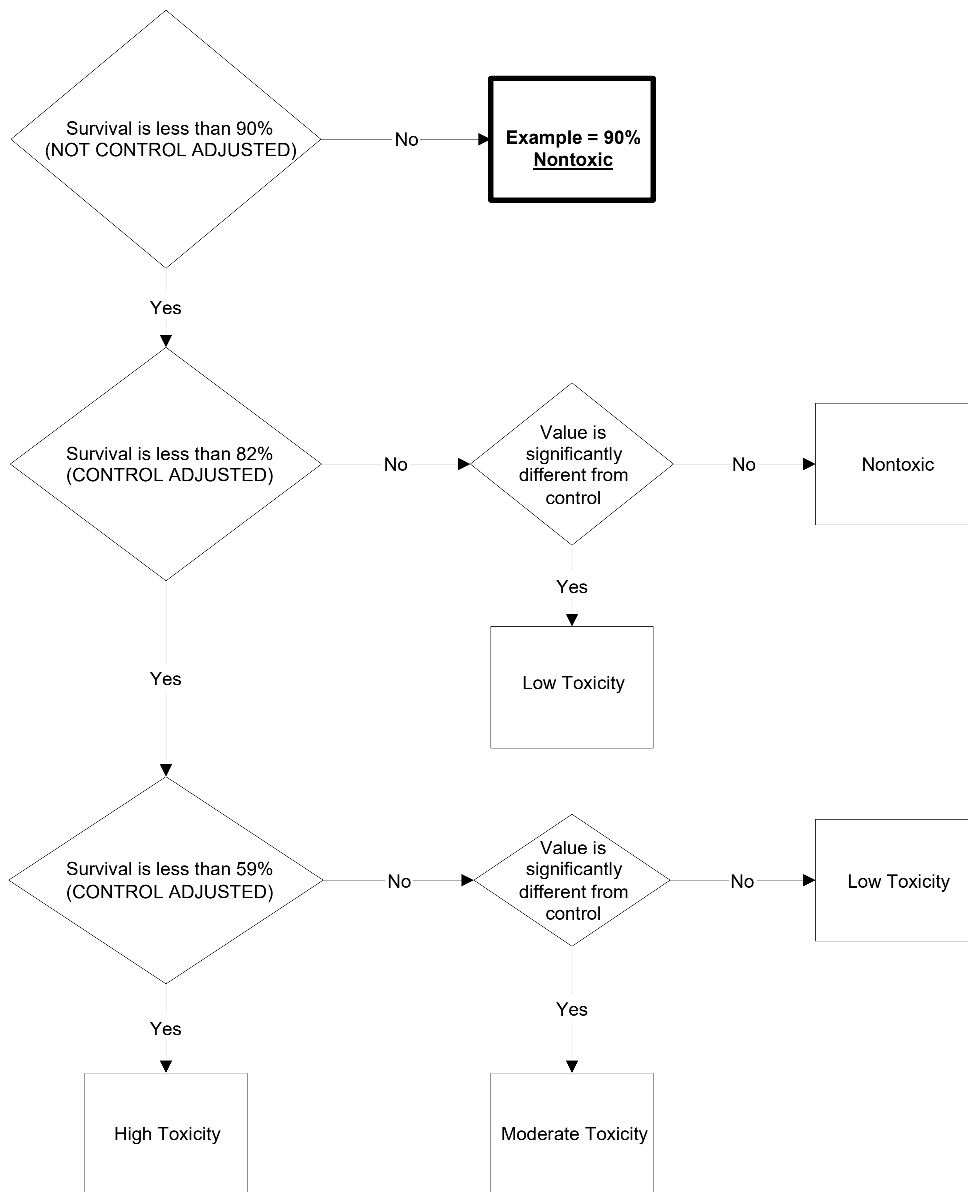


Figure 5.8. Flow chart for assignment of toxicity response category for *E. estuarius* example data.

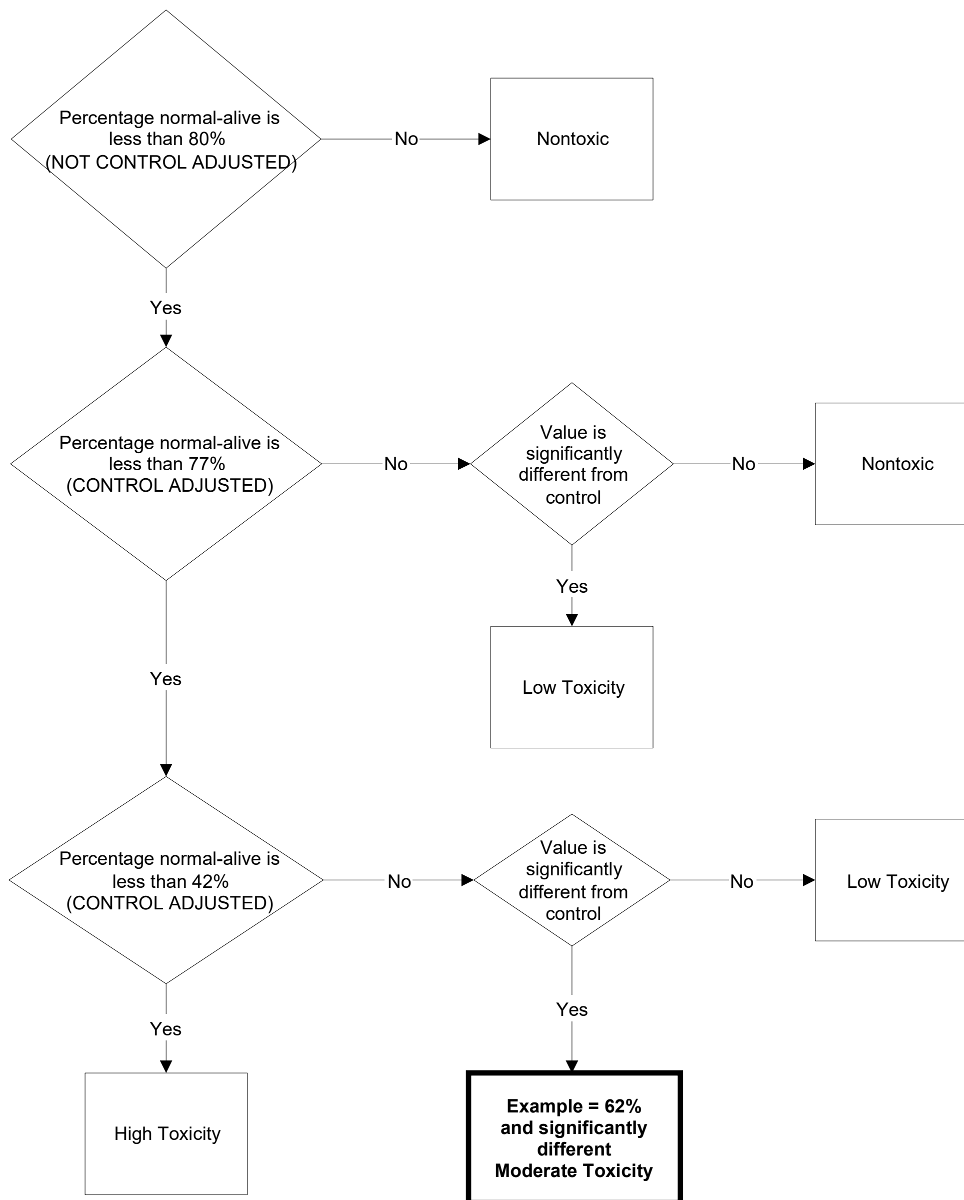


Figure 5.9. Flow chart for assignment of toxicity response category for *M. galloprovincialis* example data.

Integration of Toxicity Test Results

The final step in determining the Toxicity LOE is to integrate the toxicity test results. This is accomplished by assigning numeric category scores for each test result (Nontoxic = 1, Low Toxicity = 2, Moderate Toxicity = 3, High Toxicity = 4). The arithmetic mean of all tests corresponds to the Toxicity LOE category. Means with decimal values of 0.5 and higher are rounded up to the nearest category. Means with decimal values of less than 0.5 are rounded down.

For the example data, the *Eohaustorius* result is classified as Nontoxic (score = 1) and the *Mytilus* result is classified as Moderate Toxicity (score = 3). The mean category score for the two toxicity tests for this station is 2, which corresponds to the Low Toxicity category for the Toxicity LOE.

Chapter 6: Integrating the Lines of Evidence: Determining Aquatic Life SQO Exceedance

Objectives

Previous chapters in this manual describe methods for estuarine and marine sediment sampling and analyses of the chemistry, toxicity, and benthic community lines of evidence for sediment quality. The objective of this chapter is to describe how these lines of evidence are integrated to determine a condition assessment category for each sampling station that can be used for various monitoring and regulatory programs.

Scope

There are numerous approaches for integrating multiple lines of evidence (MLOE) data in a sediment quality assessment, and most rely at least partially on best professional judgment. This can be problematic in application to large data sets or in a regulatory setting where the assessment protocol needs to be transparent, consistent, and comparable among programs. The ALSQO integration approach described in this manual was developed for the California Sediment Quality Objectives (CASQO) program and consists of a standardized set of LOE relationships and final station assessments. The station assessments consist of five categories that describe likelihood and severity of impacts from sediment contamination on aquatic life.

Two habitat-specific integration methods are described in this chapter. One method is applicable to marine southern California and polyhaline San Francisco Bay habitats. This method utilizes the full complement of analyses and LOE categories described in previous chapters. The other method is applicable to other enclosed bays and estuaries in California, where the assessment tools for each LOE are more limited and/or more uncertain.

The ALSQO station assessment has two key limitations. First, it is relevant only for assessing impacts on aquatic life (e.g., benthic community) from direct exposure to sediment contaminants; it does not represent impacts to human health or wildlife resulting from indirect exposures as a result of the bioaccumulation and/or biomagnification of contaminants in fish and shellfish. Second, the assessment does not identify the specific chemicals causing the impacts. Additional analyses and data interpretation, known as causal assessment, are needed to identify the cause of the impacts (see Chapter 7).

Southern California Marine and San Francisco Bay Polyhaline Bays and Estuaries

Chapters 3 through 5 of this manual describe the methods for determining the Chemistry, Toxicity, and Benthic lines of evidence (LOEs), each of which are based upon multiple indices or tests (“indicators”). As described in these chapters, the indicators for each LOE are integrated to determine the overall response category for each LOE type (e.g., Nontoxic, Low, Moderate, or High for the Toxicity LOE). Four response categories are possible for each of the three LOEs, resulting in 64 possible LOE combinations. An integration framework was developed to relate each of the possible combinations to one of five final station assessment categories:

- Unimpacted

- Likely Unimpacted
- Possibly Impacted
- Likely Impacted
- Clearly Impacted

The MLOE integration framework is based on a conceptual approach that consists of two key steps (Figure 6.1). First, the category results for each LOE are combined to classify the sediment with respect to two key elements of ecological risk assessment: 1) are there adverse biological effects at the site, and 2) is chemical exposure at the site high enough to potentially result in an adverse biological response? In the second step, the biological effects and chemical exposure classification results are compared to determine the final station assessment.

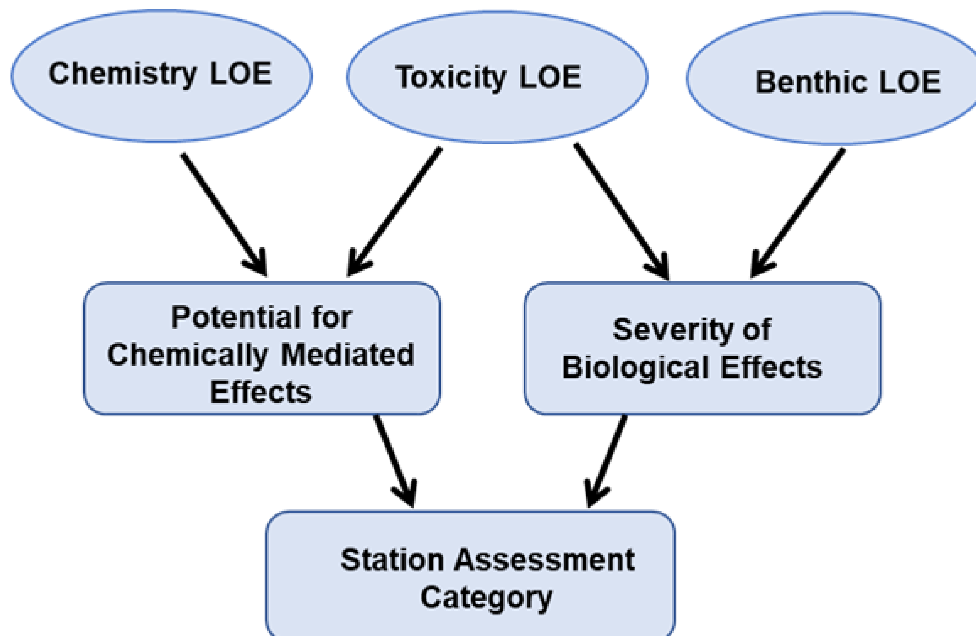


Figure 6.1. Stages of MLOE integration for ALSQO station assessment in marine and polyhaline bays and estuaries.

Details of the conceptual approach and its validation are described in the sediment quality objective (SQO) policy document (SWRCB 2008; Bay and Weisberg 2012). The efficacy of the framework was assessed by applying it to data from 25 sites throughout California and comparing the site classifications to those of six experts who provided the same data. The framework produced an answer that better matched the median classification of the experts than did five of the six experts. Moreover, the bias in response was less than that obtained from some of the experts, and the errors were relatively evenly divided between sites classified as more impacted or less impacted than the median expert classification. The framework was also applied and found to effectively distinguish sites from known degraded and reference areas within California.

In practice, determination of the final station assessment category is a simple process once the result for each LOE has been determined. The category results for the LOEs are matched to a table of all possible combinations (Table 6.1) and the corresponding final station assessment is selected. To use Table 6.1, first compile the Chemistry LOE, Toxicity LOE, and Benthic LOE results for the station sampled. Starting with the Chemistry LOE, locate the section of the table that corresponds to whether the station's sediment chemistry exposure category is Minimal, Low, Moderate, or High. Then, within the appropriate Chemistry LOE category of the table, identify the region corresponding to the Benthic LOE category for that site (i.e., Reference, Low, Moderate, or High). Finally, within the appropriate Chemistry LOE and Benthic LOE category combination, identify the Toxicity LOE category for that site (i.e., Nontoxic, Low, Moderate, or High). The row with the appropriate combination of MLOEs for a given station yields that station's sediment condition category (the final column). Additional instructions and software tools to conduct all of the required analyses and comparisons are available in the *Sediment Quality Assessment Tools* section of the *Sediment Quality* research area page at www.sccwrp.org.

Table 6.1. Station assessment categories resulting from each possible MLOE combination.

Line of Evidence Category Combination	Chemistry LOE: Sediment Chemistry Exposure	Benthic LOE: Benthic Community Condition	Toxicity LOE: Sediment Toxicity	Station Assessment
1	Minimal	Reference	Nontoxic	Unimpacted
2	Minimal	Reference	Low	Unimpacted
3	Minimal	Reference	Moderate	Unimpacted
4	Minimal	Reference	High	Inconclusive
5	Minimal	Low	Nontoxic	Unimpacted
6	Minimal	Low	Low	Likely unimpacted
7	Minimal	Low	Moderate	Likely unimpacted
8	Minimal	Low	High	Possibly impacted
9	Minimal	Moderate	Nontoxic	Likely unimpacted
10	Minimal	Moderate	Low	Likely unimpacted
11	Minimal	Moderate	Moderate	Possibly impacted
12	Minimal	Moderate	High	Likely impacted
13	Minimal	High	Nontoxic	Likely unimpacted
14	Minimal	High	Low	Inconclusive
15	Minimal	High	Moderate	Possibly impacted
16	Minimal	High	High	Likely impacted
17	Low	Reference	Nontoxic	Unimpacted
18	Low	Reference	Low	Unimpacted
19	Low	Reference	Moderate	Likely unimpacted
20	Low	Reference	High	Possibly impacted
21	Low	Low	Nontoxic	Unimpacted
22	Low	Low	Low	Likely unimpacted
23	Low	Low	Moderate	Possibly impacted
24	Low	Low	High	Possibly impacted
25	Low	Moderate	Nontoxic	Likely unimpacted
26	Low	Moderate	Low	Possibly impacted
27	Low	Moderate	Moderate	Likely impacted
28	Low	Moderate	High	Likely impacted
29	Low	High	Nontoxic	Likely unimpacted
30	Low	High	Low	Possibly impacted
31	Low	High	Moderate	Likely impacted
32	Low	High	High	Likely impacted
33	Moderate	Reference	Nontoxic	Unimpacted
34	Moderate	Reference	Low	Likely unimpacted
35	Moderate	Reference	Moderate	Likely unimpacted
36	Moderate	Reference	High	Possibly impacted
37	Moderate	Low	Nontoxic	Unimpacted
38	Moderate	Low	Low	Possibly impacted
39	Moderate	Low	Moderate	Possibly impacted
40	Moderate	Low	High	Possibly impacted
41	Moderate	Moderate	Nontoxic	Possibly impacted
42	Moderate	Moderate	Low	Likely impacted
43	Moderate	Moderate	Moderate	Likely impacted
44	Moderate	Moderate	High	Likely impacted
45	Moderate	High	Nontoxic	Possibly impacted

Table 6.1 (continued).

Line of Evidence Category Combination	Chemistry LOE: Sediment Chemistry Exposure	Benthic LOE: Benthic Community Condition	Toxicity LOE: Sediment Toxicity	Station Assessment
46	Moderate	High	Low	Likely impacted
47	Moderate	High	Moderate	Likely impacted
48	Moderate	High	High	Likely impacted
49	High	Reference	Nontoxic	Likely unimpacted
50	High	Reference	Low	Likely unimpacted
51	High	Reference	Moderate	Inconclusive
52	High	Reference	High	Likely impacted
53	High	Low	Nontoxic	Likely unimpacted
54	High	Low	Low	Possibly impacted
55	High	Low	Moderate	Likely impacted
56	High	Low	High	Likely impacted
57	High	Moderate	Nontoxic	Likely impacted
58	High	Moderate	Low	Likely impacted
59	High	Moderate	Moderate	Clearly impacted
60	High	Moderate	High	Clearly impacted
61	High	High	Nontoxic	Likely impacted
62	High	High	Low	Likely impacted
63	High	High	Moderate	Clearly impacted
64	High	High	High	Clearly impacted

Example Station Assessment for Southern California Marine and Polyhaline San Francisco Bay Habitats

The examples that were presented in Chapters 3, 4, and 5 had the following outcomes for the three LOEs:

- Chemistry LOE = ***Moderate***
- Benthic LOE = ***Low***
- Toxicity LOE = ***Low***

Applying this information to the matrix, we see that this combination corresponds to Line of Evidence Category Combination in row 38, which yields a Station Assessment (Site Condition) category of ***Possibly Impacted.*** The text in this row of the table is bold and italicized in Table 6.2, which is a subset of Table 6.1 shown for illustrative purposes.

Table 6.2. Subset of rows from Table 6.1 showing the results from the sample dataset.

Line of Evidence Category Combination	Chemistry LOE: Sediment Chemistry Exposure	Benthic LOE: Benthic Community Condition	Toxicity LOE: Sediment Toxicity	Station Assessment (Site Condition)
37	Moderate	Low	Nontoxic	Unimpacted
38	***Moderate***	***Low***	***Low***	***Possibly impacted***
39	Moderate	Low	Moderate	Possibly impacted
40	Moderate	Low	High	Possibly impacted

Interpretation of Station Assessment Results

Table 6.1 lists five station assessment categories and one additional outcome that can result from application of the CASQO assessment approach. The interpretation of these categories, in terms of certainty and magnitude of contaminated sediment impacts to aquatic life, are provided in Table 6.3. These categories reflect the reality that multiple lines of evidence may disagree and that the degree of agreement provides important information regarding the certainty of the assessment and magnitude of effects.

Table 6.3. CASQO Sediment Condition categories and interpretation.

Condition Category	Interpretation
Unimpacted	Confident that contamination is not causing significantly adverse impacts to aquatic life in the sediment
Likely Unimpacted	Contamination is not expected to cause adverse impacts to aquatic life in the sediment, but some disagreement among lines of evidence reduces certainty that the site is unimpacted
Possibly Impacted	Contamination at the site may be causing adverse impacts to aquatic life in the sediment, but the level of impact is either small or is uncertain because of disagreement among lines of evidence
Likely Impacted	Evidence of contaminant-related impacts to aquatic life in the sediment is persuasive, in spite of some disagreement among lines of evidence
Clearly Impacted	Sediment contamination at the site is causing clear and severe adverse impacts to aquatic life in the sediment
Inconclusive	No assessment category recommended. Disagreement among lines of evidence suggests that either data are suspect or additional information is needed for classification

Relationship to Sediment Quality Objectives

The categories representing the lowest estimated levels of impact to aquatic life in the sediment are *Unimpacted* and *Likely Unimpacted*. Stations classified within these two categories meet the SQO for aquatic life according to the current SQO policy. The *Possibly Impacted*, *Likely*

Impacted, and *Clearly Impacted* categories indicate, with increasing levels of severity and confidence, that sediment contamination impacts to aquatic life exist. Stations classified within these three categories do not meet the SQO for aquatic life according to the current SQO policy.

The *Possibly Impacted* station assessment is the least certain of all categorizations, and therefore requires the most caution during interpretation. Stations may be classified as *Possibly Impacted* due to low levels of effect for each LOE, indicating a low magnitude of impacts. Alternatively, a *Possibly Impacted* classification may be the result of a large disagreement between LOEs, potentially due to confounding factors or noncontaminant stressors. Repeat analyses at these stations or sampling additional locations withing the site may be useful in confirming the level of impact at these stations before deciding on management actions. Results for stations classified as *Possibly Impacted* are used in the final site assessment.

Inconclusive Results

The *Inconclusive* category is assigned when the LOE results show an extreme level of disagreement that cannot be explained by our current understanding of sediment quality assessment. An example of this situation is when a high level of toxicity is present, but there is no evidence of contaminant exposure and the benthic community shows no evidence of disturbance. A classification of *Inconclusive* should prompt review of the data and analyses associated with that station to verify the accuracy of the results. It may be useful to repeat the laboratory analyses (where feasible) or collect a repeat sample to confirm the results. Stations classified as *Inconclusive* should not be used to evaluate attainment of the SQO or the condition of a water body.

LOE Assessment and Integration for Other Bays and Estuaries

An alternative data integration method is used for enclosed bays and estuaries other than marine bays in southern California and polyhaline San Francisco Bay. The assessment approach for these other waterbodies uses the same conceptual approach and a modified set of indicators for evaluating each LOE. Variation in salinity and the relative lack of prior sediment quality triad assessments in these "other" habitats at the time of tool development and validation led to the selection of a limited and less specific group of tools for these habitats (Table 6.4). The evaluation metrics for this set of tools was modified to focus on endpoints considered to be most reliable for these habitats, such as short-term amphipod mortality and commonly applied benthic community metrics.

Table 6.4. LOE evaluation tools for other bays and estuaries.

LOE	Tool	Evaluation Metric or Index
Chemistry	Sediment concentration of Table 3.1 analytes plus other chemicals of concern (optional)	CA LRM Pmax Total sediment concentration
Toxicity	10-day survival for amphipod species appropriate for habitat (e.g., *Eohaustorius estuarius*, *Hyalella azteca*)	Percent of control survival
Benthic Community	Taxonomic analysis of benthic macrofauna	Abundance and species richness Presence/abundance of indicator taxa Presence of functional/feeding groups M-AMBI score

Assessment of each LOE for these "other" habitats results in two possible outcomes: Effect (presence of biologically significant chemical exposure, toxicity, or benthic community disturbance) or No Effect (responses within reference range or below level or of low biological significance). For indicators/indices applied in southern California marine bays and San Francisco Bay polyhaline habitats, the classification threshold for determining a LOE effect is equivalent to the threshold representing a "High" response for that indicator (Table 6.5).

Chemistry LOE assessment

The primary tool for evaluation of sediment chemistry exposure is the CA LRM, using the calculation method as described in Chapter 3 of this manual. The threshold for determining a LOE effect is the Pmax value associated with high chemical exposure (> 0.66). Optional evaluation of additional chemicals of concern not listed in Table 3.1 may also be used to evaluate the chemistry LOE, provided a threshold representing a comparable level of high exposure is available. The CSI is not used for chemical exposure evaluation because thresholds for this index are based on benthic community response relationships, which were not available for the habitats of interest.

Toxicity LOE assessment

Toxicity test methods for "other" habitats are limited to tests of 10-day amphipod survival, the most widely used and standardized type of test method. Sublethal toxicity tests were not included for this LOE because of the lack of standardized assessment methods and thresholds for some habitats at the time of method selection. The threshold for determining a toxicity LOE effect is the percent survival value associated with high toxicity from Table 5.2 for species tolerant of the habitat salinity, or an equivalent value for *Hyalella azteca* of < 62%, an alternative species tolerant of low salinity. Alternative amphipod species and thresholds approved by the Surface Water Ambient Monitoring Program (SWAMP) may also be used to evaluate the toxicity LOE.

Benthic LOE assessment

The Multivariate AMBI (M-AMBI) benthic index is recommended for classifying the benthic LOE in "other" bays and estuaries. This index is not named in the water quality control plan because development and validation were not complete at the time of the plan's adoption. However, the M-AMBI incorporates the key metrics identified in Table 6.4 and has the advantage of a numeric output that can be used to classify condition in a consistent manner that is comparable to SQO categories for other benthic indices (Table 4.27). Use of an M-AMBI score of ≤ 0.387 is recommended to represent a benthic community condition of Effect. Use of the M-AMBI is preferred over single metrics, such as species richness or indicator species presence, because the M-AMBI score integrates such metrics and has been developed to be applicable across a wide range of habitats.

Table 6.5. Numeric values and comparison methods for determination of LOE effects.

Metric	Threshold or Comparison Type
CA LRM	Pmax > 0.66
Concentration of other chemicals	Above reference range or threshold
Amphipod survival percent	< 59 (*E. estuarius*), < 62 (*H. azteca*), or SWAMP threshold
M-AMBI score	≤ 0.387
Abundance/species richness	Less than reference range or threshold
Indicator taxa presence	Outside of reference range or interval

LOE integration

The MLOE integration and station assessment approach for other bays and estuaries is like that shown in Table 6.1 for southern California marine bays and San Francisco Bay polyhaline. The availability of only two outcomes for each LOE results in eight possible LOE combinations (Table 6.6). A station is classified as Impacted with respect to the ALSQO when at least two LOEs are classified in the Effect category.

Table 6.6. Station assessment categories resulting from LOE categories for other bays and estuaries.

LOE Combination	Chemistry LOE: Category	Toxicity LOE: Category	Benthic LOE Category	Station Assessment
1	No Effect	No Effect	No Effect	Unimpacted
2	No Effect	No Effect	Effect	Unimpacted
3	No Effect	Effect	No Effect	Unimpacted
4	No Effect	Effect	Effect	Impacted
5	Effect	No Effect	No Effect	Unimpacted
6	Effect	No Effect	Effect	Impacted
7	Effect	Effect	No Effect	Impacted
8	Effect	Effect	Effect	Impacted

Use of the Assessment Results

The Aquatic Life SQO assessment framework provides a standardized and comparable description of sediment quality that can be used in a variety of applications. The current amended SQO policy document (SWRCB 2018) describes the types of intended applications and limitations. The primary anticipated uses fall into two categories: monitoring for water body assessment and receiving water limits. Assessment monitoring is typically used for applications such as regional surveys and evaluation of water bodies for listing as 303(d) impaired regions. Receiving water limits are components of various types of discharge permits and are used for regulatory purposes. The specific applications and interpretation of the results for regulatory purposes must be determined by the appropriate regulatory agencies. Requirements for regulatory applications are described in the current version of the Water Quality Control Plan for Enclosed Bays and Estuaries (SWRCB 2018).

The ALSQO assessment results may also be useful for other programs, such as the development of TMDLs. However, it is important to recognize that the ALSQO assessment does not identify the cause of impacts to the benthic community and the chemical indices making up the Chemistry LOE are not equivalent to effects thresholds for specific contaminants. The assessment results are intended to be used as a descriptor of sediment quality with respect to contaminant effects, but not as a determination of the specific cause of water body impairment.

Additional studies are often necessary to identify the cause of sediment contamination impacts and determine the appropriate actions needed to improve sediment quality. The specifics of such studies, known as causal assessment, can be varied and are often determined by many factors, including site specific conditions, types of biological effects, and the objectives of the program. Recommendations for causal assessment studies are provided in the next chapter.

Chapter 7: Causal Assessment

Sediment Toxicity

The development of tools and guidance for stressor identification is underway by several organizations and no standard guidance is yet available that addresses all aspects of the process. The following are recommendations and additional information to assist in conducting stressor identification. These recommendations are for information purposes only and do not represent regulatory requirements that are part of the SQO policy.

Three types of additional information are needed to assist in the planning of actions to improve sediment quality: 1) confirmation that pollutants are indeed the basis for the impact; 2) establishment of what specific chemical(s) is the cause of impact; 3) identification of the source of the chemical(s). The USEPA has set forth guidelines for critically reviewing data on impaired sites, listing candidate causes, characterizing the causes, and evaluating the confidence level of the identification (USEPA 2000a).

A variety of approaches are potentially useful for investigating the causes of impacted sediment quality in bays and estuaries with respect to aquatic life. All of these approaches may not be needed or appropriate for a particular investigation; the design of a study should be done on a site-specific basis. The approaches appropriate for a given waterbody or site will depend on several factors, including the magnitude and nature of the impact (e.g., toxicity or benthic community disturbance) and the suspected contaminants of concern.

Confirmation of Chemical Linkage

The MLOE assessment establishes linkage to sediment contaminants, but the lack of confounding factors (e.g., physical disturbance, non-pollutant constituents) should be confirmed. Impacts caused by physical factors at a site can be of many forms. Examples of physical stressors include reduced salinity from freshwater inputs (e.g., runoff, groundwater or wastewater discharge), impacts from dredging, very fine or coarse grain size and prop wash from passing ships. These types of stressors may produce a non-reference condition in the benthic community that is similar in appearance to that caused by contaminants. If impacts to a site are primarily due to physical disturbance, the LOE characteristics will likely show a degraded benthic community with little or no toxicity and low chemical concentrations. Supplemental information on habitat characteristics, dredging history, sediment particle size, and commercial/recreational use of the site should be evaluated if physical stressors are suspected.

There are a few sediment constituents whose presence may cause toxicity or benthic community disturbance, but that are not considered as pollutants for the MLOE assessment. These constituents, such as total organic carbon, nutrients, and pathogens, may have sources similar to chemical pollutants (e.g., wastewater treatment plant effluent) and thus produce a misleading correlation between chemical concentration and effects. Chemical and microbiological analysis will be necessary to determine if these constituents are present. The LOE characteristics for this type of stressor would likely be a degraded benthic community with possibly an indication of toxicity (e.g., due to ammonia or hydrogen sulfide), and low chemical concentrations. Supplemental data on sediment concentrations or inputs of organic carbon, nutrients, or other

non-target constituents should be reviewed if impacts due to non-pollutant stressors are suspected.

The type of impact that the SQO program is designed to identify is that caused by a significant exposure to chemical pollutants. This type of exposure would have LOE characteristics of a degraded benthic community, presence of toxicity, and elevated chemical concentrations. Depending on the level of agreement between LOE, forensic chemistry and other types of analyses may be needed to confirm that chemical exposure is the cause of impacted sediment quality. The site's chemical history should be examined to identify effluent discharges, spills or other sources of chemical contamination. Tools such as geographical information systems (GIS) and other landscape information can play a key role in that examination. It may be necessary to measure alternate suites of chemicals after more is known about a site's history. There are many types of organic chemicals that are not measured in the normal suite of priority pollutants that may be a cause of toxicity (e.g., organophosphorus and pyrethroid pesticides). Body burden data should be examined from benthic infauna living at the site (e.g., clams and worms) to indicate if contaminants are being accumulated and to what degree.

A variety of statistical methods may be helpful in confirming a linkage between chemical exposure and biological effects. Chemical-specific mechanistic benchmarks, such as those based on equilibrium partitioning of nonpolar organics (USEPA 2003a, 2008b) or binding of metals to acid volatile sulfides (USEPA 2003b) , may be used to confirm the presence of biologically significant sediment chemistry concentrations. Comparison of the sediment chemistry data to the concentrations of contaminants measured in other locations may be helpful in verifying whether there is a plausible association between specific contaminants and biological effects. An association between variations in chemical concentration and a biological effect does not indicate the cause of impacts, but such comparisons can be useful in gaining perspective on the magnitude of contamination and in prioritizing constituents for further investigation (e.g., pesticide concentrations are far below levels associated with a high probability of toxicity in other locations). Data from multiple stations within the area of interest should be examined to determine if correlations are present between measurements of sediment chemistry and biological effect.

Identification of Cause

Once it is confirmed that chemical contamination is the cause of a site's impairment, the specific chemicals or chemical groups responsible must be identified before management alternatives can be developed. A combination of approaches that include statistical, biological, and chemical analyses may be needed. These approaches fall into four general categories:

- Statistical analysis
- Laboratory toxicity identification evaluations
- Bioavailability analyses
- Confirmation

Statistical Analysis

Statistical methods include correlations between individual chemicals and biological endpoints (toxicity and benthic community). A significant correlation does not indicate a causal relationship but provides additional evidence useful for prioritizing contaminants of interest. Care must be taken when interpreting correlative relationships because individual chemicals often correlate with one another, as well as with sediment physical characteristics, such as grain size. Another statistical method is gradient analysis. For this, comparisons are made between samples collected at various distances from a potential chemical source or hotspot to examine patterns in chemical concentrations and biological responses. As the concentrations of causative agents decrease, so should biological effects.

Toxicity Identification Evaluation (TIE)

A toxicological method for determining the cause of impairment is the use of toxicity identification evaluations (TIE). During a TIE, sediment samples are manipulated chemically or physically to remove classes of chemicals or render them biologically unavailable (Figure 7.1). Following the manipulations, animal exposures are performed to determine if toxicity has been removed. These procedures are most effective if a strong and consistent toxicity signal is present. Generally, a minimum toxicity response of 25% relative to the control is needed for a productive TIE.

At the present time, there is limited detailed guidance on performing sediment TIEs. The USEPA has published guidance for some aspects of sediment TIEs (USEPA 2007). Methods for the removal of organics, metals, and ammonia from whole sediments are available from the scientific literature (Ho *et al.* 1999; Lebo *et al.* 1999; Burgess *et al.* 2000; Lebo *et al.* 2000; Pelletier *et al.* 2001; Ho *et al.* 2002; Burgess *et al.* 2003; Ho *et al.* 2004).

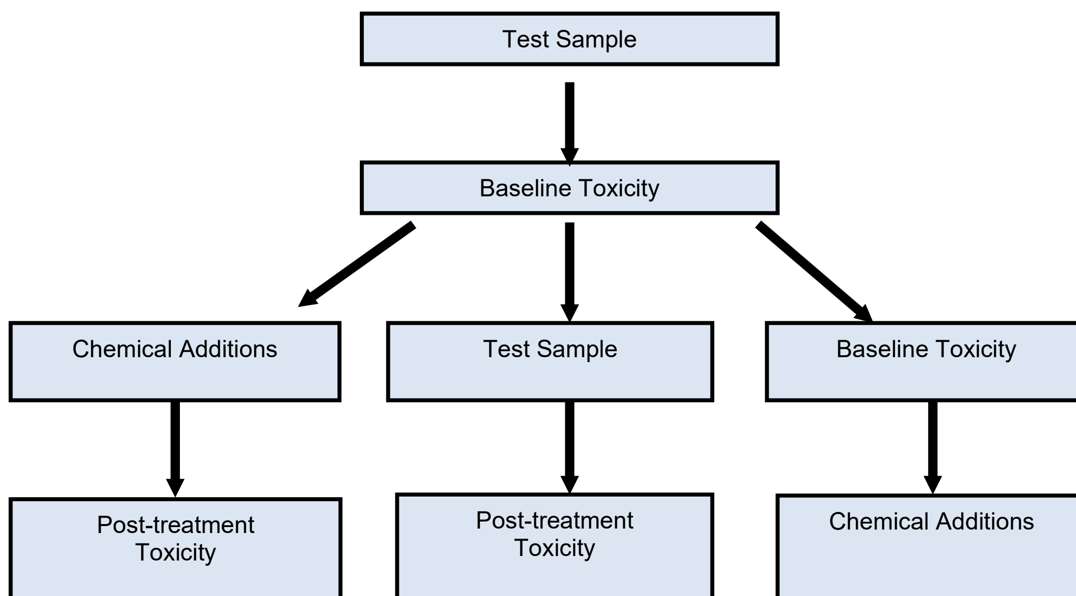


Figure 7.1. Flow chart of toxicity identification evaluation treatments.

Existing sediment TIE methods are most effective at determining cause based on broad classes of chemicals, such as metals or non-polar organics, rather than individual chemicals. Powdered coconut charcoal has been successfully used to sorb organic contaminants rendering them nontoxic (Ho *et al.* 2004). While this treatment is very effective at reducing or eliminating toxicity, due to the extremely fine nature of the charcoal it cannot be recovered from the sediment and analyzed chemically to determine which constituents it has bound. Carbonaceous, nonpolar resins have also been added to the sediment to bind organic chemicals (Kosian *et al.* 1999). While these resins are not always as effective at removing toxicity as the charcoal, they offer the advantage of being recoverable from the sediment for analysis to determine what chemicals were bound. Semipermeable membrane devices (SPMDs) have also be used to remove organic compounds from sediments (Lebo *et al.* 1999; Lebo *et al.* 2000). These devices consist of polyethylene tubing or lipid filled polyethylene tubing (known as detox spiders) that are added to the sediment. The SPMDs, like the resins, can be recovered for chemical analysis.

Cation exchange resin may be added to sediments to remove toxicity caused by cationic metals (Burgess *et al.* 2000). The cation exchange resins can be extracted with acids and the extracts analyzed to determine which metals were removed from the sediments. Prior research has shown that metals are rarely identified as the source of toxicity in whole sediment (Ho *et al.* 2002). This may be due to the higher concentrations of sulfides that are commonly associated with contaminated sediments, which bind the metals and make them biologically unavailable.

There are multiple TIE procedures for the removal of ammonia from sediments. The first is biological removal in which pieces of the alga *Ulva lactuca* are added to the overlying water (Pelletier *et al.* 2001). The algae can absorb high levels of ammonia but may also remove other contaminants. The other treatment that has been found to be equally effective is the addition of zeolite to the sediment (Burgess *et al.* 2003). This treatment has been found to also remove some cationic metals from the sample. A less effective treatment for ammonia is aeration. It has been found that aeration is not very effective at normal pH, but removes ammonia effectively when the pH is adjusted to 10 (Burgess *et al.* 2003). This treatment is also not feasible for marine systems.

Organophosphate and pyrethroid pesticides are contaminants of increasing concern in sediments. Some methods are available that are helpful for identifying toxicity caused by these classes of pesticides. Addition of the metabolic inhibitor piperonyl butoxide (PBO) to the overlying water in a sediment toxicity test chamber has been found to be effective for removing toxicity caused by organophosphorus pesticides (USEPA 1993). In the presence of pyrethroids, PBO acts as a synergist increasing toxicity over that of an untreated sample (Wheelock *et al.* 2004). If pyrethroids are suspected, an enzyme, carboxylesterase can be added, which will reduce or eliminate toxicity by rapidly degrading the pesticide. This method has only recently been used successfully with freshwater whole sediments (Phillips *et al.* 2005) and needs to be tested for marine samples.

While pore water tests are not recommended for the initial MLOE assessment of sediment quality in this program, they are a valuable tool for helping to identify the cause of toxicity (Carr and Nipper 2003). Pore water samples are amenable to all of the aqueous sample TIE methods that are available (USEPA 1996). Currently there are more tools available for the aqueous matrix than there are for whole sediment. The use of solid phase extraction columns for the removal of organics and metals is a valuable tool that is not available for use with whole sediments. These

columns can be eluted to remove the extracted chemicals, fractionated, and tested using add-back toxicity tests to provide a much finer discrimination of causative agents.

Bioavailability Analyses

Chemical contaminants may be present in the sediment but are bound to constituents rendering them not biologically available to cause toxicity or degradation of the benthic community. There are several measures of bioavailability that can be made. Chemical and toxicological measurements can be made on pore water to determine the availability of sediment contaminants (Carr and Nipper 2003). The potential bioavailability of metals can be assessed through sulfide analysis. This involves the measurement of acid volatile sulfides and simultaneously extracted metals analysis to determine if sufficient sulfides are present to bind divalent metals and maintain porewater concentrations below toxic levels (Berry *et al.* 1996). Similarly, nonpolar organic compounds can be tightly bound to sediment organic carbon, which limits bioavailability. Several methods are being developed and evaluated to assess the bioavailability of organic contaminants. These methods include solid phase microextraction (Mayer *et al.* 2000), extractions using animal digestive fluids (Weston and Maruya 2002), and weak chemical extractions (Tang *et al.* 1999).

Confirmation

After specific chemicals are identified as likely causes of impairment, analyses should be conducted to verify the results. For example, body burden analysis can be conducted on benthic infauna, such as clams and worms, living at the site. The concentrations in the animals' tissues may then be compared to established toxicity thresholds to determine if critical body residues are exceeded. Sediments can be spiked with the suspected chemicals to verify that they are indeed toxic at the concentrations observed in the field. Spiked sediment studies must be carefully designed to take into consideration the geochemistry of the site sediments, form of the contaminant, and equilibration of the contaminant among binding phases. Otherwise, the spiked sediment results may not be applicable to the study site. Alternatively, animals can be transplanted to study sites for *in situ* toxicity and bioaccumulation testing.

SECTION III: HUMAN HEALTH SQO ASSESSMENT

Chapter 8: Sediment Sampling and Chemical Analysis

The sampling and measurement of sediments for chemical contaminant concentrations is an essential part of the HHSQO framework and shares many similarities with the ALSQO framework. However, the two assessment frameworks differ substantially in the specific constituents measured and how the data are used in the assessment. For HHSQO assessment, the sediment data are used either for Tier 1 assessment or as inputs to the bioaccumulation model for the purposes of determining the Site Linkage indicator in Tier 2. Thus, the list of analytes and reporting limits differ from those used for the ALSQO framework because they are determined by the compounds measured in fish tissue, chemical exposure threshold levels and potential bioaccumulation factors.

Development of a Conceptual Site Model

For an end user to design a HHSQO assessment study, the first step is to develop a conceptual site model (CSM) that is focused on the site or waterbody characteristics, contaminants, receptors, and sources. This is needed to plan the sampling design and effects assessment. CSM development is flexible. However, CSMs generally include a written description of the specific issues associated with a site, as well as a graphical depiction of contaminant sources, processes, and receptors (i.e., target species). The graphical depiction aids in identifying potential linkages, as well as sources of uncertainty, such as what types of anglers capture and consume fish from the site, how frequently does fishing activity occur, and what seafood species occur in the site.

A key initial function of the CSM is to verify that site meets the criteria for application of the HHSQO assessment. The HHSQO and assessment framework apply to enclosed bays and estuaries only, as defined by state policy. Ocean waters, including Monterey Bay and Santa Monica Bay do not meet this definition. Application of the Tier 2 HHSQO assessment framework to inapplicable areas may result in inaccurate results, as has been shown for the Palos Verdes shelf superfund site.

The CSM should identify water body characteristics, key exposure pathways, and areas of uncertainty (USEPA 2009). For HHSQO assessment, exposure pathways are defined a priori, as human consumption of contaminated seafood. However, there are site-specific aspects of human seafood consumption that should be addressed in the CSM. Specifically, the CSM should contain information needed to determine the following parameters:

- Site boundaries and site size
- Station locations and sampling schedule
- Seafood consumer population characteristics (e.g., consumption rate)
- Seafood species to be monitored
- Food web associated with seafood species monitored

- Site-specific modification to other parameters (e.g., seafood movement range or diet) as needed
- Known or likely sources of contamination
- Fate and transport mechanisms

A definition of the site boundaries and site size is needed to aid in data collection and data reduction, in addition to being a key input for the site linkage indicator. Site boundaries may be defined based on geomorphic and hydrologic boundaries, areas of management concern, previous boundary definitions (e.g., water body segments), and other local considerations.

Selection of the site size can have a large influence on the accuracy and reliability of the assessment. Selection of a small site within a larger water body, such as an individual marina, small basin, or channel is discouraged, as it is likely that overlapping contaminant inputs from offsite sources, currents, and fish movement will substantially underestimate the influence of sediment contamination on the chemical exposure indicator. Several priority species for chemical exposure evaluation have home ranges greater than several km^2 and attempting to conduct and HHSQO assessment at sites smaller than 1 km^2 will tend to minimize the contribution of site-associated sediment contamination. In general, it is preferable to conduct the assessment at the largest scale that is relevant to the project and then consider specific management alternatives at a smaller scale if appropriate.

Another consideration is the spatial distribution of sediment contamination within a site. Some sites may contain specific areas of elevated contamination (“hotspots”), and it may be worthwhile to perform the assessment at multiple scales, including the hotspots, as well as less contaminated areas, to determine whether the assessment outcome would be different. The number and location of sediment samples should be sufficient to provide a spatially representative average concentration within the site. Since the sediment data are used to estimate the contribution of sediment contamination to seafood contamination levels, it is best for the sediment samples to represent the foraging range of the fish species monitored (generally the entire site). Use of a randomized sampling design is recommended.

The seafood consumer population is chosen based on what is known about fishing practices and consumption rates at the site. Selection of an appropriate consumer population will aid in identifying available information on local consumption rates to give perspective to the consumption rates established for determining the chemical exposure category. Surveys from other California water bodies may be employed to determine consumption rates if local data are not available. Selection of seafood species of interest will be based on the fishing and consumption practices of local consumers, as well as species known to reside in the site, and representing predominant dietary guilds.

CSM development is a dynamic process. As additional data and information becomes available, they are used to refine the CSM, by adding additional sources, pathways, or targets, or modifying existing linkages. As proposed in this framework, a preliminary CSM should be developed prior to Tier 1 assessment, and the CSM refined prior to Tiers 2 or 3 assessment.

Where and When to Sample

The recommended methods for sediment sampling for the HHSQO assessment are identical to those for the ALSQO chemistry line of evidence assessment (Chapter 2). Briefly, a grab sampler is used to obtain an undisturbed sample of the upper 5 cm of the sediment, and the sample must not be compromised by additional mixing with sediments below 5 cm during recovery of the grab sampler. A single set of sediment chemistry samples from a site should be sufficient to support both the ALSQO and HHSQO evaluations, provided the number of stations is sufficient to meet the meet the study design for each type of assessment. The trace organics chemical analysis methods needed for HHSQO assessment are compatible with those required for the ALSQO framework. However, the target analytes differ, so it is essential to give the analytical laboratory clear instructions so that the correct analyses are conducted.

At each sediment sampling location, spatial coordinates (e.g., latitude and longitude) should be recorded with a GPS monitoring device at the time the sampling device collects the sediment (i.e., is on the bottom). Care should be taken to ensure that the coordinate system is documented, to enable accurate mapping of spatial position. The following additional data regarding the sampling event and grab event should also be recorded:

- Station identification
- Date
- Time of arrival
- Collecting agency identification (or code)
- Vessel name
- System used for navigation
- Weather and sea conditions
- Salinity and temperature
- Station fail code identifying reason for abandonment (if site is abandoned)
- Time of event (grab on bottom)
- Depth of water
- Depth of penetration of grab in sediment (to nearest 0.5 cm)
- Sediment composition (e.g., coarse sand, fine sand, silt or clay, gravel, or mixed grain size; presence of shell hash)
- Sediment odor and color
- Notable observations or activities at or near the sampling site (e.g., surface sheens, vessel activity, boat cleaning, wildlife activity, etc.)

After collection, samples should be placed in pre-cleaned certified containers, using precleaned equipment. Samples should be stored immediately on ice or dry ice and should be analyzed within an appropriate holding time consistent with programmatic QAPPs. A minimum of five composite samples should be collected and analyzed in each site. For stratified sampling, at least five samples should be collected within each stratum. Quality control samples should be collected and analyzed as described in Chapter 2.

Chemical Analysis Methods

The HHSQO assessment requires measurement of sediment total organic carbon (TOC) and four classes of organochlorine contaminants: PCBs, DDTs, chlordanes, and dieldrin (Table 8.1). Target compounds for legacy pesticides follow USEPA (2000b) and OEHHA (2008).

DDTs

Six compounds comprise total DDTs and should be included in all analyses: o,p’-DDD, o,p’-DDE, o,p’-DDT, p,p’-DDD, p,p’-DDE, and p,p’-DDT.

Chlordanes

Five compounds should be included in all analyses: cis-chlordane, trans-chlordane, cis-nonachlor, trans-nonachlor, and oxychlordane.

Dieldrin

Dieldrin is an individual compound.

PCBs

Polychlorinated biphenyls (PCBs) are biphenyl compounds with between one and ten chlorine compounds attached to the phenyl groups in varying positions. There are 209 PCB compounds, individually referred to as congeners (PCB1 through PCB209), and each PCB congener has a unique IUPAC number. Monitoring programs vary in the number of PCB congeners measured, based on the study objectives. Some programs measure all 209 congeners, while most programs measure a subset of the most abundant and/or biologically active congeners. HHSQO assessment requires the measurement of 55 congeners, which is based on those congeners typically measured for California regional monitoring programs and SWAMP bioaccumulation studies. The total PCB concentration based on the sum of HHSQO congeners represents about 90% of the total PCBs in California embayments.

Measurement of additional PCB congeners in sediment may be conducted and could be useful for complementary source identification or transport studies, or for Tier 3 assessment. However, measurement of additional congeners will not affect the outcome of the Tier 2 assessment as the bioaccumulation model used in this assessment only uses data from the required 55 congeners. Potential underestimation of sediment total PCB concentration from measuring a limited set of congeners does not markedly influence the Tier 2 assessment because the total sediment PCB concentration is not used in this assessment. Underestimation of total PCB concentration could potentially impact Tier 1 assessments that are based solely on sediment chemistry, however.

Data Analysis

Sediment data should be converted to ng/g (i.e., parts per billion) dry weight prior to statistical analysis and interpretation. The site means (average) should be calculated for all constituents. This includes individual compounds in each class (e.g., PCB congeners, DDTs, and chlordane compounds) and sum of compounds. Standard error of the mean (SE) should also be calculated for sum of PCBs, sum of DDTs, sum of chlordanes, and dieldrin. SE does not need to be calculated for individual compounds.

Sediment results could be influenced by how values below detection are treated. This requires careful consideration, particularly when calculating contaminant class totals in the presence of multiple values below detection. It is recommended that undetected individual analytes be assigned a value of one-half the detection limit when used in bioaccumulation models. Nondetects are typically assigned a value of zero for the purposes of calculating sums by contaminant class. Other strategies may be used for treating nondetect data if the methods are clearly documented and used in a consistent manner for the assessment.

Methods for additional statistical analysis of the sediment chemistry data depend upon the type of HHSQO assessment conducted (e.g., Tier 1 or Tier 2). These data analysis methods are described in Chapters 11 and 12.

Table 8.1. Constituents and reporting limits (in parentheses) to be analyzed for sediment chemistry determination withing the HHSQO assessment framework. Reporting limits are on a dry weight basis.

TOC (0.1%)	**PCBs cont.**
Organochlorine Pesticides	PCB 101 (0.5 µg/kg)
cis-Chlordane (0.5 µg/kg)	PCB 105 (0.5 µg/kg)
trans-Chlordane (0.5 µg/kg)	PCB 110 (0.5 µg/kg)
cis-Nonachlor (0.5 µg/kg)	PCB 114 (0.5 µg/kg)
trans-Nonachlor (0.5 µg/kg)	PCB 118 (0.5 µg/kg)
Oxychlordane (0.5 µg/kg)	PCB 126 (0.5 µg/kg)
Dieldrin (0.2 µg/kg)	PCB 128 (0.5 µg/kg)
op-DDD (0.5 µg/kg)	PCB 137 (0.5 µg/kg)
op-DDE (0.5 µg/kg)	PCB 138 (0.5 µg/kg)
op-DDT (0.5 µg/kg)	PCB 141 (0.5 µg/kg)
pp-DDD (0.5 µg/kg)	PCB 146 (0.5 µg/kg)
pp-DDE (0.5 µg/kg)	PCB 149 (0.5 µg/kg)
pp-DDT (0.5 µg/kg)	PCB 151 (0.5 µg/kg)
	PCB 153 (0.5 µg/kg)
PCBs	PCB 156 (0.5 µg/kg)
PCB 8 (0.5 µg/kg)	PCB 157 (0.5 µg/kg)
PCB 18 (0.5 µg/kg)	PCB 158 (0.5 µg/kg)
PCB 27 (0.5 µg/kg)	PCB 169 (0.5 µg/kg)
PCB 28 (0.5 µg/kg)	PCB 170 (0.5 µg/kg)
PCB 29 (0.5 µg/kg)	PCB 174 (0.5 µg/kg)
PCB 31 (0.5 µg/kg)	PCB 177 (0.5 µg/kg)
PCB 33 (0.5 µg/kg)	PCB 180 (0.5 µg/kg)
PCB 44 (0.5 µg/kg)	PCB 183 (0.5 µg/kg)
PCB 49 (0.5 µg/kg)	PCB 187 (0.5 µg/kg)
PCB 52 (0.5 µg/kg)	PCB 189 (0.5 µg/kg)
PCB 56 (0.5 µg/kg)	PCB 194 (0.5 µg/kg)
PCB 60 (0.5 µg/kg)	PCB 195 (0.5 µg/kg)
PCB 64 (0.5 µg/kg)	PCB 198 (0.5 µg/kg)
PCB 66 (0.5 µg/kg)	PCB 199 (0.5 µg/kg)
PCB 70 (0.5 µg/kg)	PCB 200 (0.5 µg/kg)
PCB 74 (0.5 µg/kg)	PCB 201 (0.5 µg/kg)
PCB 77 (0.5 µg/kg)	PCB 203 (0.5 µg/kg)
PCB 87 (0.5 µg/kg)	PCB 206 (0.5 µg/kg)
PCB 95 (0.5 µg/kg)	PCB 209 (0.5 µg/kg)
PCB 97 (0.5 µg/kg)	
PCB 99 (0.5 µg/kg)	

Chapter 9: Water Sampling and Chemical Analysis

Water column dissolved contaminant concentrations must be measured for application of the Tier 2 HHSQO assessment framework. Tier 1 assessment does not use water column chemistry data and thus such measurements are not required for Tier 1 evaluation. The objective of the sampling and chemical analysis is to determine the site mean (average) water column concentration for each individual target compound in each class (e.g., PCBs, DDTs, chlordanes, and dieldrin). The water column data are used in the Tier 2 bioaccumulation models to determine the site linkage value.

Where and When to Sample

The location and timing of water sampling is determined by the Conceptual Site Model. Ideally, water sampling should occur at about the same time as sediment and fish sampling (e.g., within a month) so that potential variations due to temporal factors are less likely. Sampling should occur during the same index period used for other SQO sampling (June – September). A minimum of one sample, located at mid-depth within the site, is required. Location of water collection stations adjacent to sediment and fish collection stations is desirable, but not essential, as water concentrations are expected to reflect conditions throughout site due to currents and other mixing processes. Collection of samples from multiple locations is recommended so that an average value representative of the entire site can be calculated with greater confidence.

At each water sampling location, spatial coordinates (e.g., latitude and longitude) should be recorded with a GPS monitoring device at the time of sample collection (or at the time of passive sampler deployment or retrieval). Care should be taken to ensure that the coordinate system is documented, to enable accurate mapping of spatial position. The following additional data regarding the sampling event should also be recorded:

- Station identification
- Date
- Vessel name
- Collecting agency identification (or code)
- System used for navigation
- Weather and sea conditions
- Time of arrival
- Time of event (sample collection, or sampler deployment/retrieval)
- Depth of water sample or sampler deployment
- Salinity
- Water temperature

- Duration of passive sampler deployment
- Station fail code identifying reason for abandonment (if site is abandoned)

Sampling Methods

Several methods are available for collection of samples for chemical analysis. These include use of passive sampling devices (PSDs), bulk water collection and extraction, and in-situ filtration/extraction. Passive sampling is recommended for determination of dissolved contaminant concentration because it provides a time-integrated measure that is likely more representative of site conditions and the analytical methods are like those used for sediment analysis. Sampling and chemical analyses should measure the same list of analytes and achieve the recommended reporting limits, regardless of the methods used.

A passive sampler consists of an organic polymer that is held by a device and placed in contact with sediment or water for sufficient time to allow target contaminants to reach equilibrium with the sampler and other environmental phases (e.g., colloids, particles, organisms). A deployment of at least 30 days is required for measurement of dissolved organochlorines. Concentrations of target contaminants are measured by extraction of the sampler and chemical instrumental analysis. Several types of polymers/samplers have been used successfully in California, including low-density polyethylene (LDPE) sheet, known as a PED (polyethylene device) and polydimethylsiloxane (PDMS) coatings on fibers (SPME). Performance reference compounds (PRCs) should be used with all PSDs; PRCs are pre-loaded onto the sampler and provide a measure of the sampler's equilibrium with the water and are essential for accurate quantification of water concentrations. Specific methods for PSD construction, use, and analysis are varied and dependent upon the specific application. Guidance on the field and laboratory procedures for PSD use is available in USEPA/SERDP/ESTCP 2017. Given the evolving status of PSD technology, it is recommended to use of methods previously demonstrated to perform well in California enclosed embayments.

High volume water sampling is also an appropriate method for obtaining a sample for analysis of dissolved contaminants. This method typically includes collection of a water sample (by bottle or pump) that is then filtered on board or *in situ* to remove particulates, with the filtrate then passed through a resin extraction column to concentrate the analytes of interest. The column is extracted in the laboratory and analyzed using high resolution GCMS to determine dissolved chemical concentrations.

Chemical Analysis Methods

The required target analytes for Tier 2 assessment are listed in Table 9.1. The same organochlorine compounds measured in sediment and fish tissue should be measured in water so that the bioaccumulation modeling can accurately determine the influence of site contamination on fish tissue contaminant levels. Water concentration data should be converted to pg/L prior to data analysis with the SQO assessment tools.

Several additional water quality parameters are required for application of the bioaccumulation model: salinity, temperature, dissolved oxygen, and total suspended solids. Site average values for these parameters may be determined from samples collected during the sampling event (preferred) or estimated from representative measurements obtained from other sources.

Typical QC samples should be collected during field sampling and included as part of laboratory analyses. QC sample recommendations for PSDs are included in Table 9.2. Data quality objectives have not been standardized for PSD QC results and should be established as part of the study design and workplan development. Data quality objectives equivalent to those established for sediment chemistry analyses (Table 3.3) should be established where feasible.

Table 9.1. Constituents and reporting limits (in parentheses) to be analyzed in water samples.

Organochlorine Pesticides	**PCBs cont.**
cis-Chlordane (20 pg/l)	PCB 101 (1 pg/l)
trans-Chlordane (20 pg/l)	PCB 105 (1 pg/l)
cis-Nonachlor (20 pg/l)	PCB 110 (1 pg/l)
trans-Nonachlor (20 pg/l)	PCB 114 (1 pg/l)
Oxychlordane (20 pg/l)	PCB 118 (1 pg/l)
Dieldrin (20 pg/l)	PCB 126 (1 pg/l)
op-DDD ((20 pg/l)	PCB 128 (1 pg/l)
op-DDE (20 pg/l)	PCB 137 (1 pg/l)
op-DDT (20 pg/l)	PCB 138 (1 pg/l)
pp-DDD (20 pg/l)	PCB 141 (1 pg/l)
pp-DDE (20 pg/l)	PCB 146 (1 pg/l)
pp-DDT (20 pg/l)	PCB 149 (1 pg/l)
	PCB 151 (1 pg/l)
PCBs	PCB 153 (1 pg/l)
PCB 8 (30 pg/l)	PCB 156 (1 pg/l)
PCB 18 (30 pg/l)	PCB 157 (1 pg/l)
PCB 27 (30 pg/l)	PCB 158 (1 pg/l)
PCB 28 (30 pg/l)	PCB 169 (1 pg/l)
PCB 29 (30 pg/l)	PCB 170 (1 pg/l)
PCB 31 (30 pg/l)	PCB 174 (1 pg/l)
PCB 33 (30 pg/l)	PCB 177 (1 pg/l)
PCB 44 (20 pg/l)	PCB 180 (1 pg/l)
PCB 49 (20 pg/l)	PCB 183 (1 pg/l)
PCB 52 (20 pg/l)	PCB 187 (1 pg/l)
PCB 56 (20 pg/l)	PCB 189 (1 pg/l)
PCB 60 (20 pg/l)	PCB 194 (0.1 pg/l)
PCB 64 (20 pg/l)	PCB 195 (0.1 pg/l)
PCB 66 (20 pg/l)	PCB 198 (0.1 pg/l)
PCB 70 (20 pg/l)	PCB 199 (0.1 pg/l)
PCB 74 (20 pg/l)	PCB 200 (0.1 pg/l)
PCB 77 (20 pg/l)	PCB 201 (0.1 pg/l)
PCB 87 (1 pg/l)	PCB 203 (0.1 pg/l)
PCB 95 (1 pg/l)	PCB 206 (0.1 pg/l)
PCB 97 (1 pg/l)	PCB 209 (0.1 pg/l)
PCB 99 (1 pg/l)	

Table 9.2. Definitions, requirements, and frequency for typical QC samples for passive sampling. A batch is defined as 20 field samples processed simultaneously and sharing the same QC samples.

QC Sample	Definition	Frequency
	Field QC	
Field Blank	Defined as a sample of contaminant-free medium (PSD) that has been exposed to field conditions. It is used to verify that field samples are not contaminated during collection or field processing. Samples will be processed in the lab as routine field samples.	1/field deployment and recovery
Field duplicate	Field duplicates are samples of the same matrix, which are collected, to the extent possible, at the same time, from the same area, and are handled, containerized, preserved, stored, and transported in the same manner.	1/sampling event
	Laboratory QC	
Method or Procedural Blank	A combination of solvents, surrogates, and all reagents used during sample processing, processed concurrently with the field samples. Monitors purity of reagents and laboratory contamination. Matrices: PSD	1/sample batch A processing batch MB must be analyzed with each sequence.
Matrix (PSD) Spike	A PSD spiked with the analytes of interest at 10 × the MDL, processed concurrently with the field samples; monitors effectiveness of method on sample matrix. For analysis of compound classes (e.g., PCBs), the spike must contain each targeted compound class.	1/sample batch
Matrix Spike Duplicate (MSD)	The duplicate is a second matrix spike sample.	1/sample batch
Surrogate Standards	All field and QC samples are spiked with a known amount of surrogate standard just prior to extraction; recoveries are calculated to quantify extraction efficiency.	Each field and QA sample

Data Analysis

Water concentration data for individual analytes are entered into the HHSQO assessment tool for analysis using the food web bioaccumulation models. Data should be entered into the tools as pg/l. If data are available from multiple locations within the site, the average should be calculated and used for analysis. A value of one-half the detection limit should be used for analytes not detected in the analysis. If data are not available for some analytes, then an estimated value calculated based on the sediment contaminant concentration should be used. A model for estimating water column concentration from the sediment concentration is included in the HHSQO assessment tools.

Example of passive sampler application

A description of PSD use for water column sampling, based on SCCWRP PED methods, is provided to illustrate key steps and QA methods. Specific methods are included for illustration purposes and may not be applicable to other PSDs.

Low density polyethylene (LDPE) film (1 mil; 25 µm thickness) is used as the sorbing phase for highly hydrophobic compounds, due to its low cost, simplicity, and high sorption capacity. To maximize detectability in the water column, ~2 g LDPE sheet is used for each sampler that is configured in rings attached to copper wire (Figure 9.1). Triplicate samplers are recommended for each water column station.

Pre-loaded performance reference compounds (PRCs) are used to correct nonequilibrium concentrations for very hydrophobic compounds. Full equilibration of LDPE film is not feasible for all target analytes, particularly very hydrophobic compounds (e.g., PCBs with 6 or more chlorines) in a reasonable deployment period (1-2 months). Ideally, isotopically labeled analogs of target analytes are preferred to mimic actual behavior and eliminate analytical interference. However, labeled compounds are cost-prohibitive, particularly for pre-loading relatively large masses of LDPE. Hence, a suite of representative unlabeled PCB congeners that span a wide range of chlorination (and thus hydrophobicity) and that are rarely detected in the environment are used as PRCs (Table 9.3). In addition, C-13 labeled p,p'-DDE is used as a PRC for the pesticides. To ensure suitable detectability of residual PRC remaining in exposed LDPE samplers, a range of nominal pre-loading concentration (500 ~ 1000 ng PRC/g LDPE) is recommended.

Table 9.3. Recommended performance reference compounds (PRCs) for LDPE film passive samplers.

Compound	No. Cl	log K_{ow}
PCB 30	3	5.4
PCB 50	4	5.6
PCB 98	5	6.1
PCB 155	6	6.6
PCB 184	7	6.9
13C p,p'- DDE	4	6.9

To load the PRCs, LDPE sheets are soaked in a methanol:water solution (80:20, v/v) in a PRC coated glass bottle for several weeks. If a roller table is used to accelerate the loading process, soaking for fifteen days is recommended. After loading the PRCs, the PE sheets are soaked in deionized water for 24 h (×2) remove methanol from the polymer. A PRC-preloaded LDPE piece (~0.1 g) is attached on the larger non-preloaded LDPE to constitute a whole PE water column sampler.

Figure 9.1. LDPE sampler for water column.

The sampler assembly should be stored in pre-cleaned, non-contaminating containers and kept cool in the dark prior to and after deployment. The LDPE film should be handled by personnel wearing clean disposable nitrile gloves and/or solvent-rinsed stainless implements (tweezers, forceps). Exposure to ambient air and sunlight should be minimized both pre- and post-deployment.

Samplers should be deployed at the desired depth using a subsurface mooring or suspended from a structure (e.g., pier). Two or three sampler assemblies should remain stored in a freezer (-20°C) during the deployment event. These samplers should be extracted with the retrieved samplers to provide time zero reference concentrations of the PRCs. At least one additional sampler is used as travel blank for a sampling event (deployment and retrieval). The passive samplers should be kept on ice and shipped in ice chest for deployment and after retrieval.

Upon return to the laboratory, the LDPE film is rinsed with deionized water and wiped clean of visible surface residue (as needed) with a clean Kimwipe®, cut into small pieces with solvent-rinsed stainless-steel scissors and placed in a solvent rinsed glass bottle. The LDPE pieces are then spiked with recovery surrogates (e.g., DBOFB and PCB 208) and extracted three times by sonicating in dichloromethane (DCM) for 15 min ×3. The extracts are then be filtered through pre-combusted (at 500ºC) Na_2SO_4. The combined extract is concentrated and solvent exchanged to hexane. The volume of the extract should be reduced to a final volume appropriate for the expected ambient seawater concentrations and PE mass using a gentle stream of high purity N_2. After addition of internal standards (e.g., PCB30 and PCB205), extracts are analyzed by gas chromatography/mass selective detection (GC/MSD) in the selected-ion monitoring (SIM) mode following procedures described in Fernandez et al. (2012), Fernandez et al. (2014), and Joyce et al. (2015).

Freely dissolved concentration, C_{free}, is calculated based on the equilibrium partition coefficient (K_{PEW}) that should be adjusted for the temperature and salinity of the specific sampling location (Table 9.4). C_{free} is calculated from the concentration of analyte taken up by the sampler (C_{PE}), and non-equilibrium sampling correction factor (*CF*) that is derived from the fraction of PRC released (Equations 1 and 2).

$$C_{free} = \frac{C_{PE} \times CF}{K_{PEW}} \quad (1)$$

and

$$CF = \frac{1}{1 - e^{-\left[10^{(-1.04\, logKow + 5.59)}\right]Ct}} \quad (2)$$

where parameter *C* is average ratio of release rate constant of PRC from PE in the field and in the laboratory calibration (Lao et al. 2019). Based on expected analytical error and reproducibility, residual PRC concentrations < 15% and > 90% of initial concentration will likely result in estimates of CF that are subject to higher levels of uncertainty. When PRC concentrations in exposed PE are < 15% and > 90% of initial concentration, the 90/15 method is used to assign a CF value of 1 and 10, respectively.

Table 9.4. Equilibrium partition coefficients for LDPE film passive samplers.

Analyte	Kpew (L/L) for seawater @ 22 °C	Kpew (L/L) for freshwater @ 22 °C
Alpha Chlordane	387404	256545
Gamma Chlordane	414725	273124
Trans Nonachlor	838056	548622
Dieldrin	77268	52842
o,p'-DDE	1363641	872770
p,p'-DDE	1359143	865098
o,p'-DDD	347392	230261
p,p'-DDD	320571	211117
o,p'-DDT	1375349	891277
p,p'-DDT	1339031	854261
PCB (18)	197419	136016
PCB (28)	254955	172212
PCB (44)	510740	343716
PCB (49)	698933	468204
PCB (52)	541307	362780
PCB (66)	904305	596094
PCB (70)	740063	487830
PCB (74)	920710	606908
PCB (77)	895810	586160
PCB (87)	2324333	1525803
PCB (99)	2430422	1588114
PCB (101)	2292479	1498668
PCB (105)	3529500	2278837
PCB (110)	2195191	1428474
PCB (114)	3249097	2097793
PCB (118)	3563862	2291505
PCB (126)	2685719	1714986
PCB (128)	3447696	2216813
PCB (138)	6223357	3984967
PCB (149)	3125121	2015890
PCB (151)	2910750	1880204
PCB (153)	4533478	2890890
PCB (156)	3453333	2175898
PCB (157)	3534476	2227025
PCB (158)	5368063	3407357
PCB (169)	3181900	1982835
PCB (170)	3838643	2408672
PCB (177)	3316412	2099271
PCB (180)	5578500	3485920
PCB (183)	4781429	3009939
PCB (187)	5886000	3710396
PCB (189)	5255100	3231328
PCB (194)	10950667	6705645
PCB (200)	9486400	5857353
PCB (201)	6341000	3991699
PCB (206)	16379000	9896631
PCB (209)	35152806	21152417

Chapter 10: Fish Tissue Chemistry and Chemical Exposure Indicator

Measurement of contaminant concentrations in fish tissue is required for both Tier 2 and Tier 3 analyses. Use of fish tissue contaminant data is preferred for Tier 1, but not essential. Tissue contaminant data are essential for calculation of both the Chemical Exposure and Site Linkage indicators used for Tier 2 assessment and therefore the quality of these data determine the accuracy and quality of the HHSQO assessment. It is important that representative and appropriate fish species are sampled, and that the correct tissue types are analyzed. Appropriate fish species have been identified for the SQO program; these are species that reside in embayments for all/most of their life history, have a dietary connection to the sediment, and are consumed by humans. Nine primary fish species have been identified for use in the assessment; SQO assessments should use only these species where feasible, as the bioaccumulation models used for the site linkage calculations are calibrated for these species.

Where and When to Sample

Fish sampling design requires professional judgment and understanding of local site conditions, fishing practices, available collection methods, and other factors (Murphy and Willis 1996). Because of these factors, a "one size fits all" sampling program is not specified. Rather, this section describes sampling design recommendations to collect appropriate data in a variety of conditions. Local site conditions, spatial movement of finfish, and temporal variation in contaminant trends influence these recommendations.

Priority species

Tier 2 assessment requires collection of at least two species from a list of primary fish species (Table 10.1). These species reflect eight dietary guilds that reflect different food webs and potential exposure to sediment-associated contamination. The primary species include fish that are common in marine and estuarine embayments throughout California. Secondary species have also been identified, should it not be feasible to collect any of the primary species from the site (Bay et al. 2017).

Fish from at least two different dietary guilds are required for Tier 2 assessment. Inclusion of different dietary types in the assessment is intended to help ensure that the assessment is representative of the site.

Table 10.1. Priority fish species, dietary guilds, and home (foraging) ranges.

Common Name	Scientific Name	Guild	HR Basis	HR Mean	Tissue type
California halibut	*Paralichthys californicus*	Piscivore	Site length (km)	29.3	Fillet, no skin
Spotted sand bass	*Paralabrax maculatofasciatus*	Benthic diet with piscivory	Site area (km^2)	0.0071	Fillet, no skin
White catfish	*Ameiurus catus*	Benthic diet with piscivory	Site length (km)	6.9	Fillet, no skin
Queenfish	*Seriphus politus*	Benthic and pelagic with piscivory	Site area (km^2)	3	Fillet, no skin
White croaker	*Genyonemus lineatus*	Benthic without piscivory	Site area (km^2)	3	Fillet, no skin
Shiner perch	*Cymatogaster aggregata*	Benthic and pelagic without piscivory	Site area (km^2)	0.0012	Whole body, no head/tail/guts
Common carp	*Cyprinus carpio*	Benthic with herbivory	Site length x 1000 (km)	1.05	Fillet, no skin
Topsmelt	*Atherinops affinis*	Benthic and pelagic with herbivory	Site area (km^2)	0.0012	Whole body, no head/tail/guts
Striped mullet	*Mugil cephalus*	Pelagic with benthic herbivory	Site length (km)	28.2	Fillet, no skin

HR mean = mean home range of seafood species under consideration (km or km^2, depending on taxa).

Sampling location

Fish are mobile and cannot be expected to reside in a fixed location. Therefore, seafood collection need not occur at individual sediment stations. Collection effort should focus on characterizing potential human exposure throughout the site. In consideration of this, sampling location selection should consider where human fishing activity is expected to be high (e.g., public piers) and where target species are likely to be caught.

Fish move and forage across large areas, and therefore will be exposed to contamination at relatively large spatial scales. Fish sampled at a specific location will indicate contaminant exposure in the region surrounding that location. Because of this, it is appropriate to sample a subset of areas over a spatial region.

Sampling site locations should be considered during the conceptual site model development. Factors to consider in site selection include areas targeted by anglers, sites where sediment-associated species are likely to occur, spatial patterns in sediment contamination, habitat, depth, morphometry (e.g., subregions, channels, or harbors), and sampling access.

The appropriate number of sampling locations is related to the size of the site being assessed. For large sites (e.g., estuarine or marine embayments greater than 8 km^2 in area), OEHHA recommends that sampling should target multiple locations within the site, to better characterize

the range of seafood exposure conditions (Gassel and Brodberg 2005), as well as possible spatial patterns in exposure that may be associated with areas of elevated contamination. For moderately sized sites (e.g., harbors, estuarine subregions, or small marine embayments, less than 8 km^2 in area), all finfish sampling can be performed in one location. In these situations, pooling of samples across the entire site would be acceptable.

Sampling of small sites (e.g., small harbors or estuarine creeks) less than 1 km^2 in area) is not recommended. Such sites are smaller than the foraging area of most of the priority species and the target species may not be resident or readily captured within the site. In this situation, fish sampling should be constrained to species with small foraging ranges (e.g., shiner perch, spotted sand bass, topsmelt, and carp). It may be necessary to collect fish at nearby locations, where captured fish may be expected to exhibit some exposure to the site. Similarly, data or samples obtained by other studies from locations near the site may be used. Best professional judgment should be used when deciding whether to use tissue samples obtained from outside of small sites. Factors to consider should include number of samples needed to reduce uncertainty, sampling difficulty, and whether the samples are likely to be representative of the site.

Seasonal and temporal variation

Timing of fish tissue collection should be considered. Sampling should be conducted during the SQO index period used for other sampling, June – September, to facilitate integration with other data types. However, fish show changes in contaminant content with season, often associated with seasonal changes in lipid content or reproductive activity. Concentrations of chlorinated organic contaminants are generally elevated when tissue lipid contents are highest (Greenfield *et al.* 2005).

The CSM and sample design should account for the possibility of seasonal variation in contaminant concentrations. To be protective, if seasonality is known, sampling could occur when lipid content is expected to be highest. This is typically just prior to reproductive activity. If sufficient resources are available, fish could be sampled from multiple seasons.

Tissue samples that represent current conditions should be used in the assessment. Many legacy contaminants, including organochlorine pesticides and PCBs, have shown declines over past decades. It is preferred that fish collection occur in the same year as sediment and water samples collection. In general, only data collected in the past five years should be used for assessment.

Compositing

Assessment of seafood contamination should be based on sampling a statistically representative population of fish, which can be achieved through sufficiently large sample sizes. Using composites of multiple individuals for laboratory analysis can increase the representativeness of chemical concentration exposure. In compositing, tissue samples from multiple individuals of the same species are combined prior to chemical analysis.

Composite samples should meet the following four requirements:

1. Individuals should be from the same fish species
2. Individuals should be from the same general collection location and collection event

3. Individuals should have similar body sizes
4. Tissue mass should be the same for each fish included in the composite

If the body size of fish targeted by seafood consumers varies widely, composites may be size stratified. With respect to size, OEHHA recommends following USEPA's 75% guideline: that the smallest individual in the sample should be no less than 75% of the total length of the largest individual (Gassel and Brodberg 2005). Each composite should consist of tissue from a minimum of three individual fish, with five individuals preferred (Gassel and Brodberg 2005).

When composites are prepared, OEHHA recommends that the tissue from each fish should be weighed and subsampled to achieve even mass for each organism. This preparation method will ensure that each member of the composite contributes equally to the composite concentration (Gassel and Brodberg 2005). Composites are composed of fillet or whole-body tissue, depending on the species (Table 10.1).

Analysis of individual fish may be employed as an alternative to compositing. However, individual analysis will not improve the ability to characterize the average tissue concentration, upon which the HHSQO assessment framework is based. To accurately characterize average chemical concentrations in seafood, a larger number of laboratory analyses must be employed when using individual fish samples than when using composites. For example, the accuracy of the average estimate will be about the same for three composite analyses of five fish each versus fifteen individual analyses. But analysis of individuals will increase the ability to describe the full range of variability in fish concentrations. Analysis of individuals will also aid in understanding potential factors contributing to elevated concentrations, such as lipid content, size, or sampling location.

Sample size

A minimum of three samples (preferably composites) should be collected and analyzed for each target species at each site to be evaluated for HHSQO assessment. If more fish are obtained in field sampling, the number of individuals per composite can be much larger. For example, the Regional Monitoring Program for San Francisco Bay routinely analyzes 20 individuals per shiner perch composite (Greenfield *et al.* 2003).

For the HHSQO evaluation, triplicate composite samples should be obtained from a minimum of two priority list species (Figure 10.1). Each species must be from a separate feeding guild.

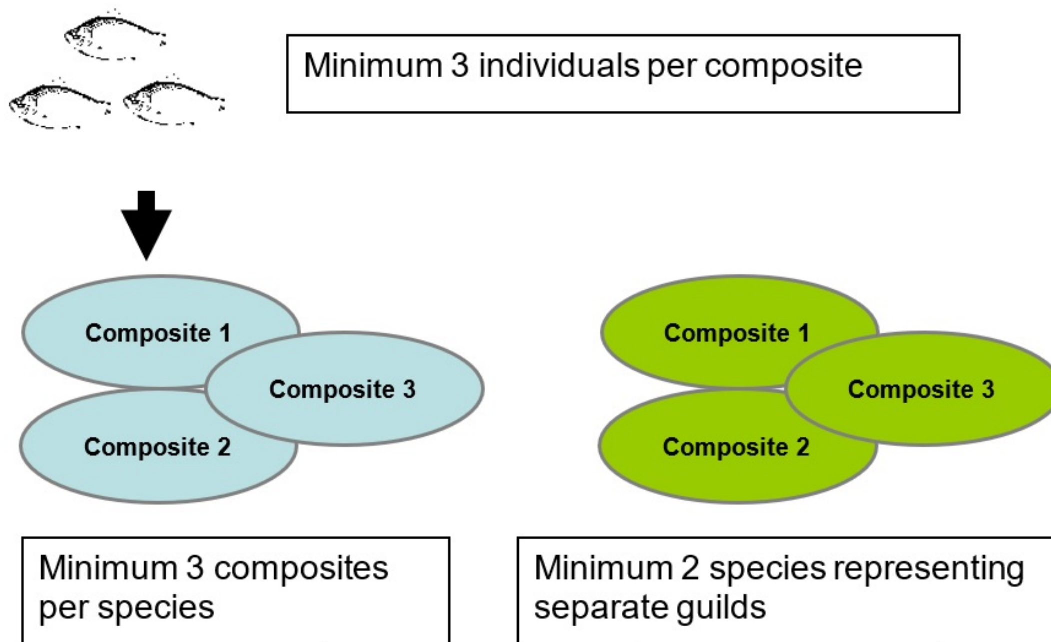


Figure 10.1. Recommended minimum sample sizes for Tier 2 assessment.

Field data for fish sampling

At each sampling location, spatial coordinates (e.g., latitude and longitude) should be recorded with a GPS monitoring device. In addition, all fish samples should be measured in the field for total length (longest length from tip of tail fin to tip of nose/mouth), fork length (longest length from fork of tail fin to tip of nose/mouth), and body mass, to confirm legal capture size. Total length analysis is particularly important, as this is the method of evaluating whether fish meet legal size requirements.

Sampling methods

A variety of sampling methods may be employed, depending on what is most suitable for collecting target species (Murphy and Willis 1996). Sampling methods for marine fish typically include gill or fyke nets, trawling, beach seines, and hook and line. In low salinity estuarine zones, electrofishing may also be appropriate.

Sampling should target seafood that may be legally caught and consumed by humans. Thus, all samples should be within the legal range for capture and consumption. Fish length should be measured and compared to CDFW legal fishing sizes to determine whether fish samples are appropriate as human prey. Legal fishing size information may be obtained from the CDFW website http://www.dfg.ca.gov/. If sufficient legal-sized fish cannot be collected, then sublegal fish may be used, provided that all scientific collection regulations are complied with.

Preservation of sample integrity

Regardless of method used, effort should be made to not puncture the skin of the fish or otherwise damage the tissue until dissection (Gassel and Brodberg 2005). Sources of extraneous

tissue contamination should also be avoided, and cleaning measures should be taken to reduce exposure. Potential contaminant sources include grease from boat winches or cables, engine fuel spills and exhaust, dust, and ice. Wrapping samples in precleaned aluminum foil or Teflon® sheeting and storage in waterproof plastic bags is an appropriate method to minimize contamination. Dissection and fillet preparation should be performed in a laboratory cleanroom environment, rather than in the field.

Sample Preparation

Preparation of tissue, sample compositing, and homogenization should be conducted in a laboratory cleanroom environment whenever possible. USEPA (2000b) recommended protocols for organic sample preparation should be followed. These include processing samples using stainless steel, anodized aluminum, borosilicate glass, polytetrafluroethane (PTFE), quartz, or ceramic equipment. Fillet preparation should be performed on PTFE or glass cutting boards using instruments composed of corrosion resistant stainless steel, quartz, titanium, or PTFE. Prepared samples should be stored in borosilicate glass, quartz, or PTFE containers with PTFE-lined lids (USEPA 2000b).

Fish tissue type

Tissue type and preparation can significantly influence contaminant concentrations in fish. Concentrations are typically higher for whole body than fillet tissue due to the inclusion of lipid rich tissue. Two types of tissue samples are analyzed for HHSQO assessment, depending upon the species (Table 10.1). Muscle fillet, without the skin, is analyzed for larger fish typically consumed as a fillet or steak (e.g., halibut, white croaker, and spotted sand bass). The whole body, excluding the head, tail, and guts, is analyzed for smaller fish that are typically consumed whole.

Chemical Analysis Methods

Tissue should be analyzed for the same suite of chlorinated pesticides and PCB congeners as specified for sediments (Table 8.1). The same reporting limits utilized for sediment should also be attained for tissue. Except for possible tissue-specific methods of homogenization, extraction, and cleanup, chemical analysis methods for tissue are generally the same as those used for sediment analysis.

Each tissue sample must also be analyzed for percent total lipids using solvent extraction. Tissue lipid content is an important parameter that influences contaminant levels in fish and is needed for SQO bioaccumulation modeling. Generally, lipids are determined by gravimetric analysis of a small subsample of the tissue solvent extract used for chemical analysis.

Data Management and Reporting

Tissue data should be converted to ng/g (parts per billion) wet weight prior to entry into the HHSQO assessment tool. The site mean (average) total concentration should be calculated from the sum of each compound category (e.g., PCBs, DDTs, chlordanes, and dieldrin). Total contaminant concentration for a contaminant type is calculated as the sum of all individual detected compounds in that category. Nondetected analytes are treated as zero for summation

purposes. In the case where all compounds in a given category are nondetectable, then the sum is usually assigned a value equal to the highest detection limit of any of the class components.

Separate results should be calculated for each fish guild monitored. The standard error of the mean (SE) should also be calculated for each guild. Means and SE do not need to be calculated for individual compounds in each class.

Calculation of Chemical Exposure Indicator

Human exposure and health risk associated with consumption of contaminated seafood is influenced by the risk of both cancer and noncancer adverse effects. California's Office of Environmental Health Hazard Assessment (OEHHA) has established statewide seafood consumption guidelines that consider both cancer risk and noncancer hazard, balanced by the health benefits of consuming fish (OEHHA 2008). These guidelines are in two forms: the Fish Contaminant Goal (FCG) that represents a contaminant concentration below which no significant adverse health effects are expected, and an Advisory Tissue Level (ATL) that represents a concentration range where no significant health effects are expected at specified consumption rates of 1, 2, or 3 meals per week.

Chemical exposure is determined using the weighted average measured tissue concentration (Figure 10.2). This is calculated for each chemical class based on the consumer diet proportion for each fish species represented and measured tissue concentration. This weighted average is compared to the chemical exposure thresholds, which are based on the FCG and ATLs (Table 10.2).

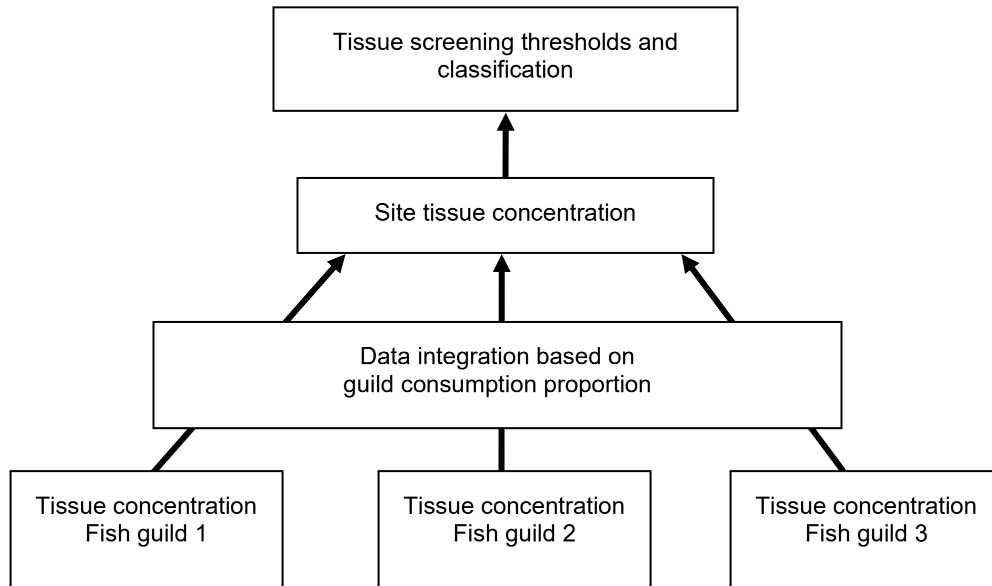


Figure 10.2. Steps for determining the chemical exposure to seafood consumers. The number of guilds included in the analysis depends on the conceptual site model.

Table 10.2. Chemical Exposure thresholds. Based on OEHHA Fish Contaminant Goals (FCGs) and Advisory Tissue Levels (ATLs) (OEHHA 2008). All values given in ng/g (ppb) wet weight.

	Chemical Exposure Category				
Contaminant	Very Low[1]	Low[2]	Moderate[3]	High[4]	Very High[5]
Chlordanes (ng/g)	< 5.6	5.6 to < 190	190 to < 280	280 to < 560	≥ 560
DDTs (ng/g)	< 21	21 to < 520	520 to < 1000	1000 to < 2100	≥ 2100
Dieldrin (ng/g)	< 0.46	0.46 to < 15	15 to < 23	23 to < 46	≥ 46
PCBs (ng/g)	< 3.6	3.6 to < 21	21 to < 42	42 to < 120	≥ 120

[1] Equivalent to FCG.
[2] Equivalent to ATL3; 3 servings per week.
[3] Equivalent to ATL2; 2 servings per week.
[4] Equivalent to ATL1; 1 serving per week.
[5] Equivalent to OEHHA no consumption guideline.

Chapter 11: Site Linkage Indicator

Site Linkage is a unitless parameter that describes the relative strength of the influence of site sediment contamination on fish tissue contaminant levels within the site. This parameter reflects the confidence we have that sediment contamination is the major source of the contaminant bioaccumulation in fish from the site. A high site linkage indicates that site sediment contamination, including the associated flux into the water column and food web bioaccumulation, is likely the primary source of fish contamination. Low site linkage indicates that offsite factors, such as other sources of contamination or perhaps fish movement patterns have a dominant influence on the bioaccumulation measured in fish collected from the site.

Site linkage is calculated as the ratio of tissue bioaccumulation estimated from bioaccumulation models using site sediment to the tissue contaminant level measured in fish collected from the site. High site linkage is assumed for Tier 1 assessment and is not calculated. Calculation of the site linkage indicator is required for Tier 2 and Tier 3 assessments. This section describes the methods used for calculating site linkage in Tier 2.

Bioaccumulation Modeling Approach

Site linkage calculations are based on the Arnot and Gobas food web bioaccumulation model (Arnot and Gobas 2004), modified by Gobas and Arnot (2010). This is a mechanistic bioaccumulation model which has limited complexity to increase ease of application while accurately depicting the primary bioaccumulation processes. The model is structured to depict contaminant concentration in biota as the mass balance of several key uptake and loss processes. The model accounts for uptake by diet and respiration; loss by egestion, metabolism, and respiratory elimination; and apparent loss through growth dilution, as represented by the following equation:

Biota Concentration (C_{Biota})=

(Respiratory Uptake*Water Concentration+ Dietary Uptake*Prey Concentration) /

(Elimination + Fecal Egestion + Growth + Metabolism)

This concentration is then used to calculate the Biota Sediment Accumulation Factor (BSAF) as the ratio between the estimated tissue concentration and that in the sediment for each contaminant category:

$$BSAF = C_{Biota}/C_{Sed}$$

where C_{Sed} is the measured concentration in the sediment.

Detailed information on the model and its application for use in the HHSQO assessment framework can be found in Bay et al. 2017.

Calculation of Site Linkage Score

Site linkage is evaluated differently in each assessment tier. High site linkage is assumed in Tier 1, in line with the simplified and conservative approach of this tier. Tier 2 evaluates site linkage using a standardized approach and bioaccumulation model, where the estimated biota concentration resulting from site sediment contamination is compared to observed concentrations for the same species (Figure 11.1). Evaluation of site linkage in Tier 3 may use alternate methods or models, if the conceptual approach and method of classifying linkage strength is comparable to Tier 2.

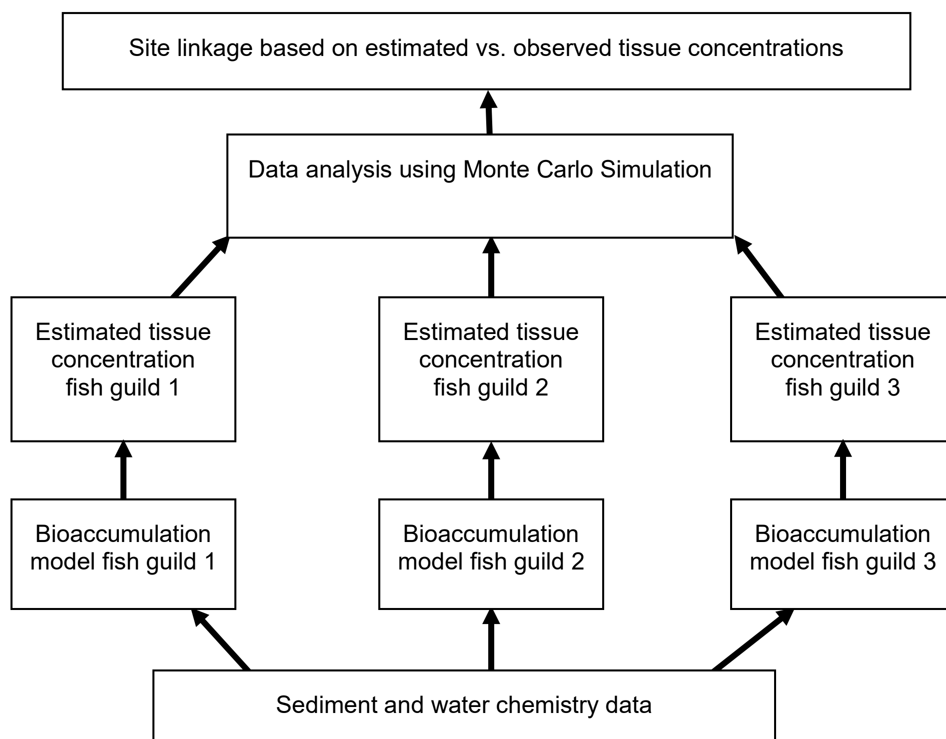


Figure 11.1. Steps for determining the site linkage to fish bioaccumulation. The number of guilds included in the analysis depends on the conceptual site model.

Monte Carlo Simulation (MCS) is used to incorporate the variability of both the measured sediment and tissue concentrations, the fish guild home range (HR), and the estimated BSAF values. For this analysis, a lognormal distribution is used for BSAF and sediment concentrations. The values and statistical distributions for each home range is shown in Table 11.1. A total of 10,000 iterations should be used for the MCS.

The overall site linkage is calculated as:

Site linkage = C_{Est}/C_{Tis}

Where:

C_{Est} = weighted average estimated tissue concentration based on the proportion of the human diet for each guild (ng/g). The diet proportion for each guild is assumed to be equal in the absence of site-specific consumption data; and

C_{Tis} = weighted average observed tissue concentration based on the proportion of the human diet for each guild (ng/g), calculated using a lognormal distribution for measured mean tissue data and standard error for each guild for total chlordanes, total dieldrin, total DDTs, and total PCBs. The weighted average for each contaminant class is based on the proportion of the human diet for each guild (ng/g). The diet proportion for each guild is assumed to be equal in the absence of site-specific consumption data.

The average estimated tissue concentration for each guild, i, and contaminant class (i.e., total DDTs) is calculated using the following equation:

$C_{Est,i} = \Sigma C_{Sed} \times SUF_i \times BSAF_i$

Where:

ΣC_{Sed} = lognormal distribution of sediment concentration using the measured mean and standard error;

Site Use Factor (SUF_i) = HR distribution using the HR mean and HR standard deviation (SD) as found in Table 11.1. If the calculated SUF is less than 1, use the calculated value. If the SUF is greater than 1, use the value of 1; and

$BSAF_i$ = lognormal distribution of the mean BSAF for guild, i, from the model prediction and the calculated BSAF SD.

BSAF SD = CVBSAF*BSAF

Where:

CVBSAF = 0.782

The CVBSAF was estimated from empirical data using the following equations:

$$SD = \sqrt{(m^2)(e^{\sigma^2} - 1)}$$

$$CV = \frac{\sqrt{(m^2)(e^{\sigma^2} - 1)}}{m} = \sqrt{(e^{\sigma^2} - 1)}$$

Where:

σ = lognormal standard deviation;

m = mean (this value cancels out); and

CV = coefficient of variation

Table 11.1. Home range parameters for each sport fish guild.

Species	Guild	HR Basis	HR Mean	HR SD	HR Distribution
California halibut	Piscivore	Site length (km)	29.3	60	Lognormal
Spotted sand bass	Benthic diet with piscivory	Site area (km^2)	0.0071	0.0073	Lognormal
White catfish	Benthic diet with piscivory	Site length (km)	6.9	9.6	Lognormal
Queenfish	Benthic and pelagic with piscivory	Site area (km^2)	3	4.689	Lognormal
White croaker	Benthic without piscivory	Site area (km^2)	3	4.689	Lognormal
Shiner perch	Benthic and pelagic without piscivory	Site area (km^2)	0.0012	0.000804	Lognormal
Common carp	Benthic with herbivory	Site length*1000 (km)	1.05	9904	Inverse gamma cumulative*
Topsmelt	Benthic and pelagic with herbivory	Site area (km^2)	0.0012	0.000804	Lognormal
Striped mullet	Pelagic with benthic herbivory	Site length (km)	28.2	80.34	Lognormal

HR mean = mean home range of seafood species under consideration (km or km^2, depending on taxa).

HR SD = standard deviation of home range of seafood species

*Inverse gamma cumulative distribution requires 3 terms: Probability= a random number uniformly distributed over $0 \le x < 1$; Alpha= HR mean value (shape parameter); Beta= HR SD value (scale parameter)

Use of Monte Carlo Simulations for the site linkage calculation results in a distribution of values. A site linkage value of 0.5 is used as the threshold for categorization. The site linkage category is based on the cumulative percentage of the distribution that falls above the threshold (Table 11.2). Two examples of classification of this distribution are shown in Figure 11.2.

Table 11.2. Site linkage categories for Tier 2 assessment.

Cumulative % of site linkage distribution above threshold	Linkage threshold	Outcome
0-25%	0.5	1. Very Low
26-50%	0.5	2. Low
51-75%	0.5	3. Moderate
76-100%	0.5	4. High

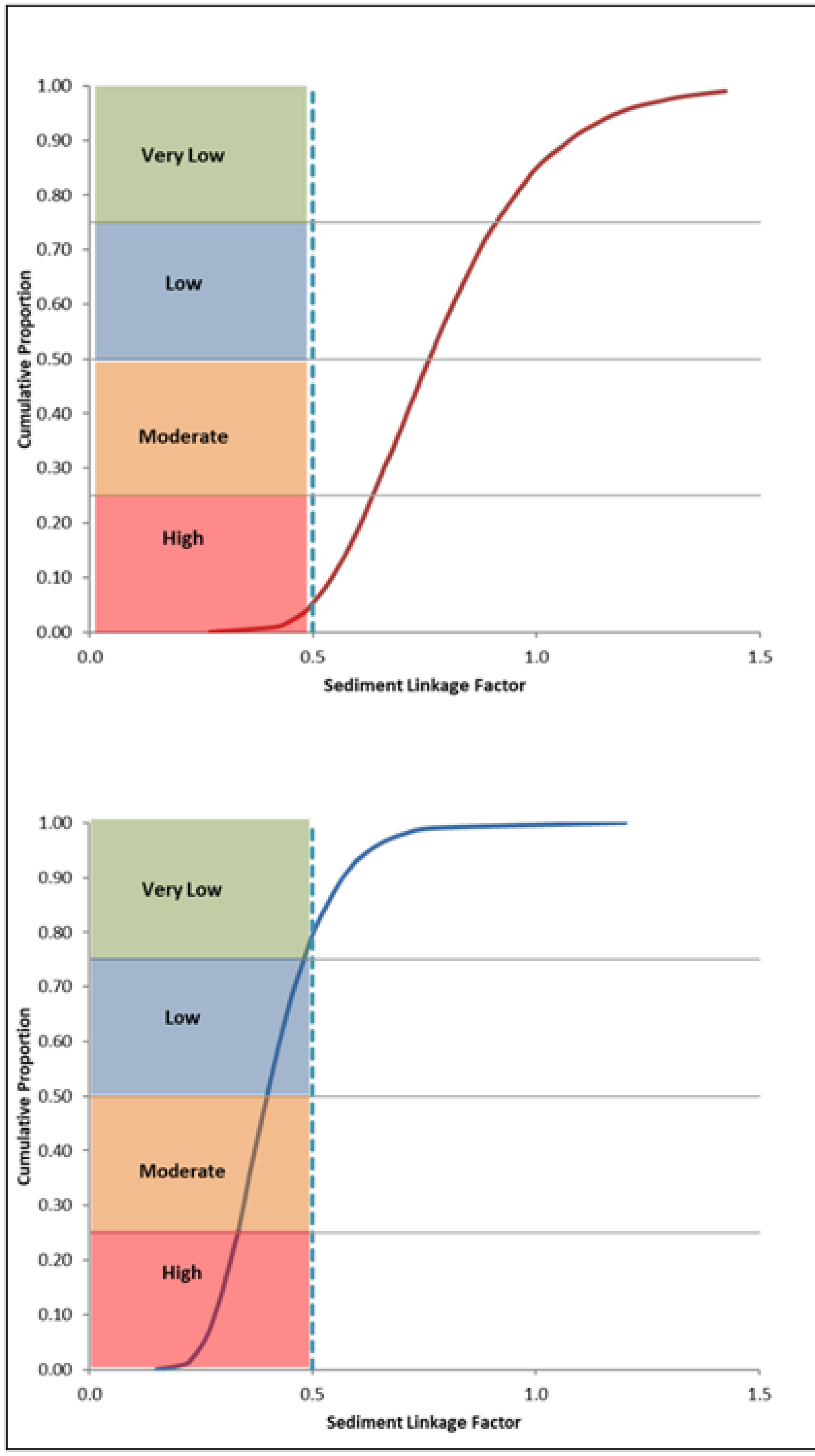


Figure 11.2. Examples of site linkage distributions indicating High (top) and Very Low (bottom) linkage categories. The blue dashed line is the 0.5 threshold and the colored sections denote the classification distribution ranges.

Site Linkage Interpretation

Sediment organochlorine contamination is known to be a significant factor in food web contamination and a likely contributor to contamination of sportfish tissue body burdens. Under simple and straightforward site conditions (e.g., assessment of entire embayment, adequate data set, no significant external sources of contamination, and fish movement and diet match model assumptions), site linkage is expected to be strong (Moderate or High categories) and be the predominant source of tissue contamination. This should be the outcome in most assessment scenarios that are not complicated by additional complexity or uncertainty associated with factors such as small spatial scale, nonrepresentative data, other exposure sources, strong contamination gradients, or variable fish life history characteristics.

Unexpected or counterintuitive linkage estimates have occurred in some applications of the framework, especially cases where limited or incomplete data on fish or sediment were available for assessment. Both high linkage values (e.g., > 5) or unexpected low linkage categories (e.g., Very Low or Low for a site with relatively high sediment PCB contamination) have been observed.

The occurrence of unusual linkage values should prompt careful review of the study design, data quality/representativeness, and data analysis steps. Unexpectedly high linkage values could indicate that the SQO bioaccumulation model is not appropriate for the site being assessed and that a Tier 3 assessment may be informative. However, such values could also be the result of data analysis errors, incomplete sediment/tissue data, or data that is not spatially representative of site contamination. For example, unusually high linkage values have resulted during assessment of sites with small-scale gradients of sediment contamination such that the sediment data reflected high PCB contamination near the site margins while fish tissue contamination reflected the larger site area which had lower overall sediment contamination. Careful development of a study design and conceptual site model that considers such sources of variability may reduce uncertainty and inflation of the linkage estimates.

Unexpectedly low linkage values occur less frequently. Low linkage values may be accurate and reflect the presence of significant external contamination sources for the fish that are not reflected in the sediment chemistry (e.g., urban runoff or fish foraging outside off site). But low linkage may also indicate deficiencies in the study design or data set used for the assessment, such as when contamination gradients are present and the sediment and fish tissue data do not represent the same portion of the site. For example, use of fish tissue data collected from contamination hotspots within the site (e.g., contaminated marshes or harbors) paired with sediment chemistry data from a less contaminated portion of the site would result in an apparent low level of linkage. In such cases, the spatial sampling design and data selection should be reviewed for a possible mismatch and it may be helpful to collect additional samples for chemical analysis.

Chapter 12: Data Integration: Determining Human Health SQO Exceedance

Three tiers of data analysis and integration are available for assessment of the HHSQO (Table 12.1) The tiers vary in terms of their intended use and data requirements. While the same general types of chemistry data (e.g., sediment, water, tissue) are used in each assessment, the data requirements and analysis methods vary. A separate assessment analysis is conducted for each contaminant group (PCBs, DDTs, chlordanes, or dieldrin).

Tier 1 is intended for a screening evaluation of a site, often using data available from other monitoring programs. This level of assessment is intended to identify clean sites that meet the HHSQO and thus do not require more detailed analysis. Because the data requirements and analysis complexity are low, Tier 1 uses conservative assumptions to provide a margin of safety.

Tier 2 is a standardized assessment framework that produces outcomes that are comparable on a statewide basis and indicate whether the HHSQO is met or exceeded. Application of Tier 2 is required for HH SQO evaluations when the Tier 1 results indicate that a full assessment is needed. Chemistry data for sediment, fish tissue, and water are required for Tier 2. Bioaccumulation modeling combined with Monte Carlo Simulation is used to determine the site linkage indicator.

Tier 3 is an optional site-specific assessment that may use data types or analysis methods that differ from Tier 2. Use of Tier 3 is triggered by the need to accommodate site-specific conditions or make site management decisions that cannot be accomplished using Tier 2. The methods and data used for this tier are determined in consultation with the regulatory authority.

This section describes the methods and gives examples for conducting Tier 1 and 2 assessments. The requirements and characteristics of Tier 3 assessments are also described. Specific methods for Tier 3 assessment are not provided because the study design is determined on a site-specific basis.

Table 12.1. HHSQO assessment Tier comparison.

Assessment Type	Purpose	SQO Outcomes	Required Data Types	Data Integration Method
Tier 1	Site screening	SQO met: Full assessment needed	Sediment and/or tissue	Specified
Tier 2	Standardized assessment	SQO met; SQO not met	Sediment, tissue, and water	Specified
Tier 3	Site-specific assessment; Management planning	SQO met; SQO not met	Sediment, tissue, other data as needed	Approved workplan

Tier 1

Tier 1 Screening Evaluation

Tier 1 is an optional rapid screening evaluation that uses available data. The outcome of this assessment is binary, either the site is classified as unimpacted (meets HHSQO) or the site is determined to have sufficient potential for human health impacts and thus a complete assessment is needed (Tier 2).

Tier 1 utilizes conservative assumptions to address uncertainty and reduce the chance of concluding unacceptable chemical exposure does not exist when in fact it does. High site linkage is assumed for Tier 1, meaning that all the observed fish tissue contamination is assumed to be derived from site sediment contamination. The assessment outcome is therefore based on whether resident fish tissue contamination exceeds a screening threshold. The assessment may be based on either measured fish tissue chemistry or sediment contaminant concentration, depending upon what data are available (Figure 12.1). If both sediment and tissue contamination data are available, the Tier 1 assessment is performed using both data types. A separate assessment is conducted for each contaminant group (total PCBs, total DDTs, total chlordanes, or dieldrin).

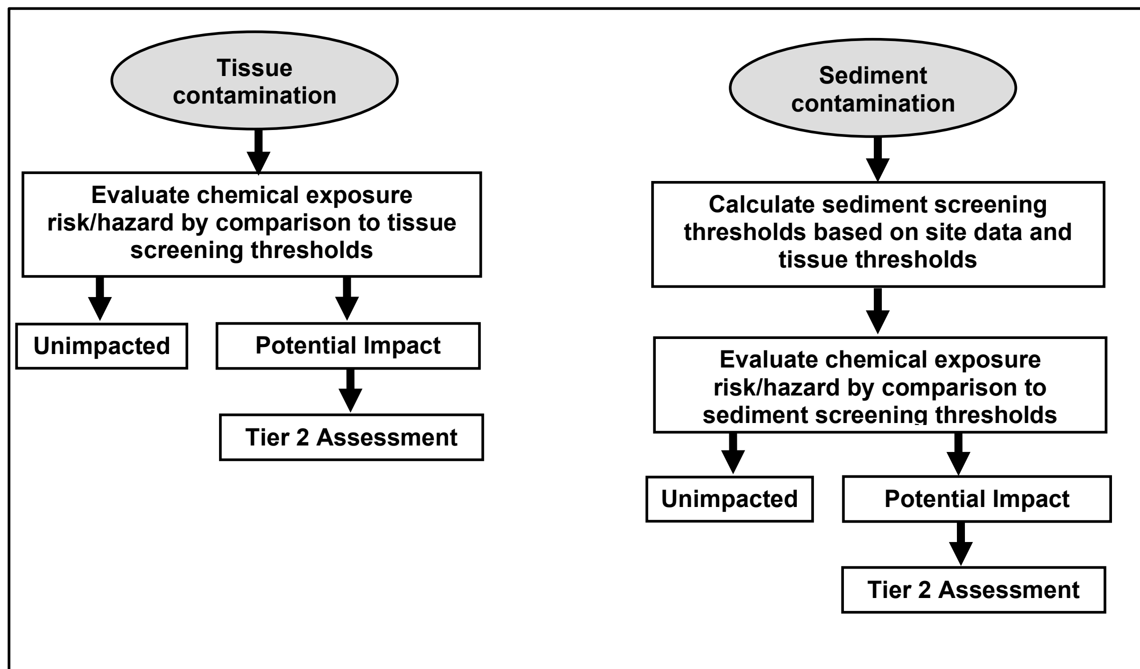


Figure 12.1. Tier 1 assessment steps using either fish or sediment contamination data.

Tier 1 Tissue Evaluation

The tissue-based chemical exposure evaluation is performed by comparing measured tissue concentration to screening thresholds. This comparison is based on tissue data from all the

species identified in the CSM. The 95% upper confidence limit (UCL) of the concentration is used for comparison to provide a safety factor and address data uncertainty.

The Tier 1 tissue evaluation concentration (C_{Tis95}) is equal to the mean of the 95% upper confidence limit (UCL) of the mean tissue concentration for each species.

$$C_{Tis95} = [\Sigma\ C_{Tis95i}]/n$$

Where

C_{Tis95i} = 95%UCL of the mean tissue concentration for sport fish species i (ng/g ww)

Σ is the sum across all species, and n is the number of species.

If the sample size is too low to calculate the UCL for a given species (less than 3), the maximum concentration is used for that species.

Chemical exposure is evaluated by comparison of C_{Tis95} to screening thresholds corresponding to the maximum of the OEHHA ATL3 range (Table 12.2). If the tissue concentration is equal to or greater than any tissue screening threshold in Table 12.2, there is the potential for unacceptable chemical exposure and a Tier 2 evaluation is required. If the tissue concentration is less than the tissue screening threshold, the chemical exposure is acceptable, and the site is assessed as Unimpacted.

Table 12.2. Tier 1 tissue screening thresholds (maximum of ATL3).

DDTs (ng/g ww)	PCBs (ng/g ww)	Chlordanes (ng/g ww)	Dieldrin (ng/g ww)
520	21	190	15

Tier 1 Sediment Evaluation

Tier 1 sediment evaluation is based on estimated chemical exposure from the sediment. This evaluation is performed by comparing site sediment concentration to sediment screening thresholds. Sediment screening thresholds are calculated for each contaminant group based on the TOC of the sediment and the BSAF for target species. The sediment evaluation is conducted by comparing the 95% UCL of the site sediment contamination to the threshold. If the sample size is too low to calculate the UCL, the maximum sediment contaminant concentration is used for comparison to the threshold.

The sediment threshold (T_{Sed}) for each contaminant group is calculated as the Tier 1 tissue threshold (Table 12.2) divided by a biota-sediment accumulation factor (BSAF):

$$T_{Sed} = (T_{Tis})/(BSAF)$$

Where

T_{Sed} = sediment screening threshold (ng/g dw)

T_{Tis} = tissue screening threshold (ng/g ww)

BSAF = biota-sediment accumulation factor (BSAF)

The highest BSAF for the dietary guilds identified in the CSM is used in calculating the sediment screening threshold. The BSAF for each contaminant is determined based on the dietary guild, and site sediment TOC (Table 12.3). A site sediment concentration (95% UCL of the mean) that is less than T_{Sed} is classified as Unimpacted. Sediment concentrations equal to or greater than T_{Sed} are classified as potentially impacted and require Tier 2 evaluation to make a HHSQO assessment.

Table 12.3. Tier 1 biota sediment accumulation factors by fish dietary guild and corresponding priority species.

TOC (%)	Piscivore California Halibut				Benthic with Piscivory Spotted Sand Bass				Benthic with Piscivory White Catfish			
	Chlordanes	DDTs	Dieldrin	PCBs	Chlordanes	DDTs	Dieldrin	PCBs	Chlordanes	DDTs	Dieldrin	PCBs
0.1	65.8	83.1	28.1	79.0	68.6	90.8	28.5	86.8	86.6	118.3	34.1	113.6
0.2	33.7	43.6	14.2	41.8	35.7	48.5	14.5	46.9	44.9	63.2	17.3	61.4
0.3	23.0	30.4	9.5	29.4	24.7	34.4	9.8	33.6	31.0	44.8	11.6	43.9
0.4	17.6	23.7	7.2	23.1	19.2	27.3	7.5	26.8	24.0	35.5	8.8	35.1
0.6	12.2	17.0	4.8	16.8	13.7	20.1	5.2	20.0	17.0	26.1	6.0	26.1
0.8	9.5	13.6	3.7	13.5	10.9	16.4	4.0	16.5	13.5	21.3	4.6	21.5
1.0	7.9	11.5	3.0	11.6	9.3	14.2	3.3	14.3	11.4	18.4	3.8	18.6
1.2	6.8	10.1	2.5	10.2	8.1	12.7	2.8	12.9	9.9	16.3	3.2	16.7
1.4	6.0	9.1	2.2	9.2	7.3	11.5	2.5	11.8	8.9	14.8	2.8	15.2
1.6	5.4	8.3	1.9	8.4	6.7	10.7	2.2	10.9	8.1	13.7	2.5	14.1
1.8	5.0	7.7	1.7	7.8	6.2	10.0	2.0	10.2	7.5	12.8	2.3	13.2
2.0	4.6	7.2	1.6	7.3	5.8	9.4	1.9	9.7	7.0	12.0	2.1	12.4
2.5	3.9	6.2	1.3	6.4	5.1	8.3	1.6	8.6	6.1	10.6	1.7	11.0
3.0	3.4	5.6	1.1	5.7	4.6	7.5	1.4	7.8	5.5	9.6	1.5	10.0
3.5	3.1	5.1	1.0	5.2	4.2	7.0	1.3	7.2	5.0	8.8	1.3	9.2
4.0	2.8	4.7	0.9	4.8	3.9	6.5	1.2	6.7	4.6	8.2	1.2	8.6

Table 12.3. (Continued).

TOC (%)	Benthic and Pelagic with Piscivory Queenfish				Benthic without Piscivory White Croaker				Benthic and Pelagic without Piscivory Shiner Perch			
	Chlordanes	DDTs	Dieldrin	PCBs	Chlordanes	DDTs	Dieldrin	PCBs	Chlordanes	DDTs	Dieldrin	PCBs
0.1	89.0	110.6	37.2	103.9	71.7	85.6	42.7	82.4	27.6	32.9	15.9	31.6
0.2	45.1	56.7	18.7	53.6	37.9	47.3	21.8	46.2	14.3	17.6	8.0	17.2
0.3	30.4	38.7	12.5	36.8	26.6	34.4	14.8	34.0	9.9	12.5	5.4	12.3
0.4	23.1	29.7	9.4	28.3	20.9	27.9	11.3	27.9	7.6	9.9	4.1	9.8
0.6	15.8	20.7	6.3	19.9	15.2	21.2	7.8	21.5	5.4	7.3	2.8	7.3
0.8	12.1	16.2	4.8	15.6	12.3	17.7	6.1	18.1	4.3	6.0	2.2	6.1
1.0	9.9	13.5	3.9	13.1	10.6	15.6	5.0	16.0	3.6	5.1	1.8	5.3
1.2	8.5	11.6	3.2	11.4	9.4	14.1	4.3	14.5	3.2	4.6	1.5	4.7
1.4	7.4	10.3	2.8	10.1	8.5	12.9	3.8	13.4	2.8	4.2	1.3	4.3
1.6	6.6	9.3	2.5	9.2	7.8	12.0	3.5	12.5	2.6	3.8	1.2	4.0
1.8	6.0	8.5	2.2	8.4	7.3	11.3	3.2	11.8	2.4	3.6	1.1	3.7
2.0	5.5	7.9	2.0	7.8	6.9	10.7	2.9	11.2	2.2	3.4	1.0	3.5
2.5	4.6	6.8	1.6	6.7	6.1	9.5	2.5	9.9	1.9	3.0	0.8	3.1
3.0	4.0	6.0	1.4	6.0	5.5	8.7	2.2	9.1	1.7	2.7	0.7	2.8
3.5	3.6	5.4	1.2	5.5	5.1	8.0	2.0	8.3	1.6	2.5	0.6	2.6
4.0	3.2	5.0	1.1	5.0	4.7	7.4	1.8	7.8	1.5	2.3	0.6	2.4

Table 12.3. (Continued).

	Benthic with Herbivory Common Carp				Benthic and Pelagic with Herbivory Topsmelt				Pelagic with Benthic Herbivory Striped Mullet			
TOC (%)	Chlordanes	DDTs	Dieldrin	PCBs	Chlordanes	DDTs	Dieldrin	PCBs	Chlordanes	DDTs	Dieldrin	PCBs
0.1	62.0	63.7	43.3	59.2	20.7	22.3	14.0	21.0	44.3	36.9	40.7	33.4
0.2	32.6	34.1	22.5	32.0	10.6	11.6	7.0	11.0	23.3	19.7	21.1	18.0
0.3	22.7	24.2	15.6	22.9	7.2	8.0	4.7	7.6	16.3	14.0	14.6	12.9
0.4	17.8	19.2	12.1	18.3	5.5	6.2	3.6	5.9	12.7	11.1	11.3	10.3
0.6	12.8	14.2	8.6	13.6	3.8	4.4	2.4	4.2	9.2	8.2	8.1	7.7
0.8	10.3	11.6	6.8	11.2	2.9	3.5	1.8	3.4	7.4	6.8	6.4	6.4
1.0	8.8	10.0	5.8	9.8	2.4	2.9	1.5	2.9	6.3	5.9	5.4	5.6
1.2	7.8	8.9	5.1	8.8	2.1	2.6	1.3	2.5	5.6	5.3	4.8	5.0
1.4	7.0	8.2	4.6	8.0	1.8	2.3	1.1	2.3	5.1	4.8	4.3	4.6
1.6	6.5	7.5	4.2	7.4	1.7	2.1	1.0	2.1	4.7	4.5	3.9	4.3
1.8	6.0	7.0	3.9	7.0	1.5	1.9	0.9	1.9	4.4	4.2	3.6	4.1
2.0	5.6	6.6	3.6	6.6	1.4	1.8	0.8	1.8	4.1	4.0	3.4	3.9
2.5	4.9	5.9	3.2	5.8	1.2	1.6	0.7	1.6	3.7	3.6	3.0	3.5
3.0	4.5	5.3	2.9	5.3	1.1	1.4	0.6	1.4	3.3	3.3	2.7	3.3
3.5	4.1	4.9	2.6	4.9	1.0	1.3	0.5	1.3	3.1	3.1	2.5	3.0
4.0	3.8	4.5	2.5	4.5	0.9	1.2	0.5	1.2	2.9	2.9	2.3	2.9

Tier 1 Interpretation

The Tier 1 screening evaluation is applied to assess whether sediment is unimpacted in relation to the sediment quality objective or if a more detailed analysis is required by conducting a Tier 2 assessment. Possible outcomes of the Tier 1 screening are:

- If only tissue is evaluated in Tier 1 and the result is equal to or greater than the threshold for any constituent, **Tier 2 is needed** for those constituents. If the tissue concentration is below the threshold, then sediment quality is **Unimpacted** for that constituent.
- If only sediment is evaluated in Tier 1 and the result is equal to or greater than the threshold for any constituent, **Tier 2 is needed** for those constituents. If the sediment concentration is below the threshold, then sediment quality is **Unimpacted** for that constituent.
- If both tissue and sediment data are evaluated and both results fall below the thresholds, then sediment quality is **Unimpacted.**
- If both tissue and sediment data are evaluated and the tissue results fall below the threshold, but the sediment equals or exceeds the threshold, then sediment quality is **Unimpacted.**
- If both tissue and sediment data are evaluated and the sediment results fall below the threshold, but the tissue equals or exceeds the threshold, then sediment quality is potentially unacceptable, and a **Tier 2 assessment is needed.**
- If both tissue and sediment data are evaluated and both results equal or exceed the thresholds, then sediment quality is potentially unacceptable, and a **Tier 2 assessment is needed.**

Tier 1 Site Assessment Steps

The following steps should be followed to conduct a Tier 1 screening assessment:

Step 1: Develop a conceptual site model

The conceptual site model is needed to define the site boundaries, guide selection of fish species to evaluate, and identify appropriate chemistry contamination data for analysis.

Step 2: Calculate contaminant concentration

For either fish tissue or sediment data, the contaminant concentration is calculated as the 95% upper confidence limit (UCL) of the arithmetic average. The estimated concentration is obtained using all appropriate data within the site boundaries (defined in Step 1). For sediment data, average total organic carbon (TOC) concentration is also calculated.

Step 3: Calculate sediment threshold for site

For sediment data evaluation, a sediment threshold is determined for each contaminant class at the site. The sediment threshold is calculated as the tissue threshold divided by a

bioaccumulation factor (BSAF), as described previously. The BSAF is obtained from a look up table, based on the contaminant, fish guild, and site TOC. Round down to the next lowest value in the table for TOCs between table rows.

Step 4: Compare data to thresholds and determine assessment outcome

The results are interpreted as described above. A Tier 1 assessment results in one of two categorical outcomes, depending on how site concentrations compare to threshold values.

1. **Unimpacted:** Concentrations are below threshold values, indicating low potential risk to sport fish consumers based on the data evaluated. Results should be corroborated with both data types, if available. If only one data type is available, then no further evaluation is needed, and the Tier 1 assessment is complete.

2. **Tier 2 assessment needed:** Concentrations are equal to or above threshold values, indicating potential unacceptable sediment quality. Tier 2 assessment is needed to confirm the results.

Tier 1 Assessment Example

The Tier 1 assessment process is illustrated in the following example:

Step 1. The CSM identified three fish species for assessment: leopard shark, white croaker, and shiner perch.

Step 2. Measurements of sediment and fish tissue were compiled and analyzed to calculate the 95% UCL (Table 12.4).

Table 12.4. Summary of case study sediment and fish tissue data. All contaminant results are the 95% UCL of the average. Tissue values are reported as ng/g ww and sediment values are reported as ng/g dw.

Matrix	Dietary Guild	DDTs	PCBs	Chlordanes	Dieldrin	TOC (%)
Leopard shark	Benthic diet with piscivory	10.5	25.3	1.4	0.7	
White croaker	Benthic diet without piscivory	70.4	251	12.1	2.2	
Shiner perch	Benthic and pelagic diet without piscivory	27.4	122	6.7	1.6	
Three species combined	Average of three species	36.1	133	6.7	1.5	
Sediment		2.6	7.0	0.2	0.1	1.3

Step 3. Sediment thresholds were calculated based on Tier 1 tissue thresholds and the highest BSAF corresponding to the three fish species sampled (Table 12.3). If the exact TOC is not listed in the table, then either use the value for the next lowest TOC or interpolate. In this example BSAFs corresponding to 1.2 % TOC were used. For all compounds, white croaker (benthic diet without piscivory) had the highest BSAF. These BSAFs were used to calculate the sediment thresholds (Table 12.5).

Table 12.5. Calculation of Tier 1 sediment thresholds for the example case study based on white croaker BSAF.

Compound	Tissue Threshold	BSAF	Sediment Threshold Calculation	Sediment Threshold
DDTs	520	14.1	520/14.1 =	36.9
PCBs	21	14.5	21/14.5 =	1.4
Chlordanes	190	9.4	190/9.4 =	20.2
Dieldrin	15	4.3	15/4.3 =	3.5

Step 4. Tissue and sediment results were compared to the Tier 1 threshold for each contaminant (Table 12.6). Examination of the tables illustrates consistent findings for sediment and tissue. Neither sediment nor tissue results exceeded the Tier 1 thresholds for DDTs, chlordanes, or dieldrin. Both sediment and tissue concentrations exceeded their respective thresholds for PCBs. In this example, both sediment and tissue data indicated that the sediment quality is unimpacted for chlordanes, DDTs and dieldrin, and that a Tier 2 evaluation should be conducted for PCBs.

Table 12.6. Comparison of tissue and sediment concentrations to the Tier 1 screening thresholds. Highlighted results exceed the Tier 1 tissue threshold and sediment threshold (based on white croaker BSAF).

Parameter	DDT	PCB	Chlordane	Dieldrin
Observed tissue concentration	36.1	133	6.7	1.5
Tissue threshold	520	21	190	15
Observed sediment concentration	2.6	7.0	0.2	0.1
Sediment threshold	36.9	1.4	20.2	3.5

Tier 2

Tier 2 is a standardized site-specific assessment intended for use with data collected based on the developed conceptual site model. There are five possible outcomes of this assessment: Unimpacted, Likely Unimpacted, Possibly Impacted, Likely Impacted, and Clearly Impacted. Outcomes of Possibly, Likely, and Clearly Impacted represent a failure to meet the HHSQO.

The assessment category result for Tier 2 is based on a comparison of the Chemical Exposure and Site Linkage indicator categories (Figure 12.2). Tier 2 uses site-specific chemistry data combined with a set of standardized thresholds and food web bioaccumulation models to calculate the indicator results. Several measures of data variability and uncertainty are also included in the calculation to provide a more representative assessment. The methods for determining these indicator categories are described in Chapters 10 and 11.

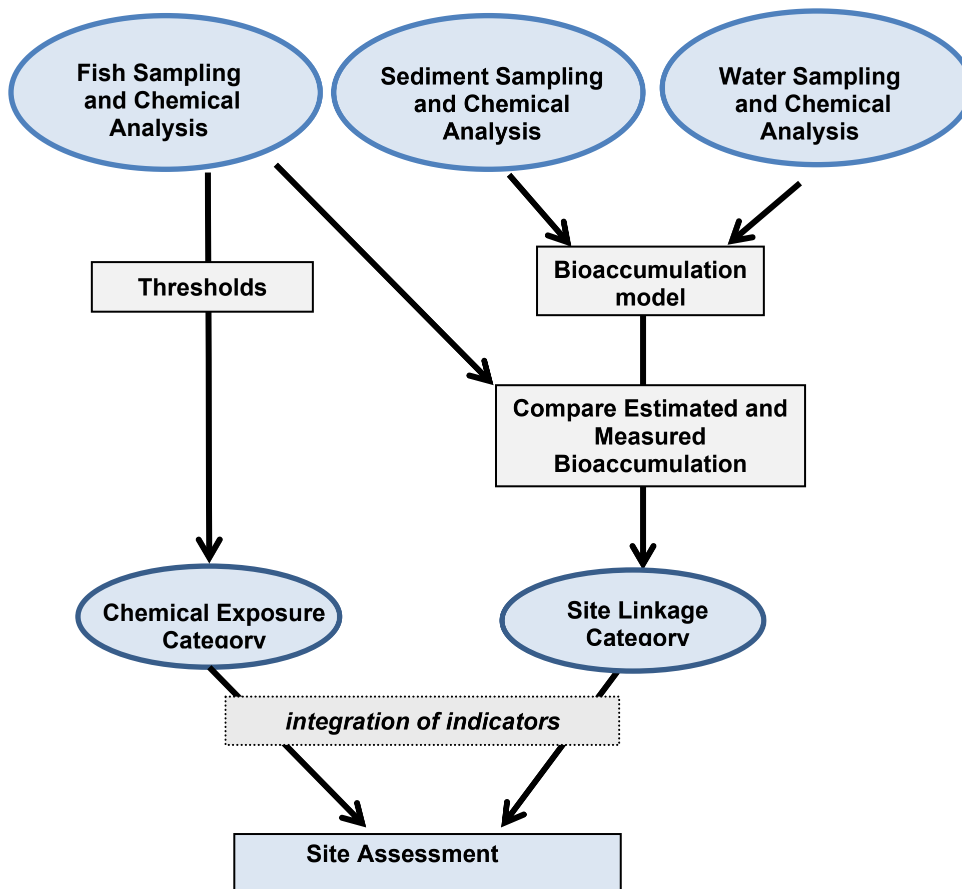


Figure 12.2. Calculation, integration, and interpretation of Tier 2 assessment indicators.

As for Tier 1, a separate assessment is conducted for each contaminant group (PCBs, DDTs, chlordanes, or dieldrin). These calculations can be performed using the equations and bioaccumulation model described in the HHSQO assessment technical report (Bay et al. 2017). A Decision Support Tool (DST) has been developed to facilitate data analysis and integration. The DST is a set of linked Excel worksheets that conduct the model and indicator calculations necessary to complete the Tier 2 assessment. The DST and a user guide are available at: http://www.sccwrp.org/Data/DataTools/SedimentQualityAssessment.aspx.

An online Tier 2 analysis tool is also available at https://sccwrp.shinyapps.io/sqo_web/ or from the collection of sediment quality analysis tools on the SCCWRP web site: http://www.sccwrp.org/Data/DataTools/SedimentQualityAssessment.aspx.

Both the DST and online tool will provide equivalent results that conform to the Tier 2 assessment requirements. The DST is run locally on a computer and requires the installation of a special Excel add-in to enable all the required statistical calculations. Chemistry and supporting data are entered into the DST worksheets manually by the user. All the bioaccumulation model equations are visible in the DST and the results of several related calculations are produced for additional context (e.g., cancer risk and noncancer hazard indices).

Use of the online HHSQO assessment tool requires an internet browser and an internet connection. Data is entered into the tool by uploading a series of data files. The format and variable names of the data files must match the requirements of the online tool for the analyses to run successfully. Results from the online tool can be downloaded and saved for reporting and further analysis.

Tier 2 Interpretation

The Chemical Exposure and Site Linkage results are compared and interpreted using a set of standardized logic rules to determine the final site assessment category. Each indicator result is classified into one of multiple categories (five Chemical Exposure categories and four Site Linkage categories) resulting in 20 possible combinations (Table 12.7). Each pair of indicator categories is assigned one of five possible assessment categories: Unimpacted, Likely Unimpacted, Possibly Impacted, Likely Impacted, and Clearly Impacted. Assessment categories of Unimpacted and Likely Unimpacted indicate that the HHSQO is met. All other categories indicate exceedance of the HHSQO. Repeat sampling or collection of additional samples or fish species is recommended if there is a desire to confirm a site assessment of Possibly Impacted.

The chemical exposure threshold separating the Low and Moderate categories defines the presence of unacceptable contaminant exposure to humans from consuming locally caught seafood. Chemical Exposure categories of Very Low and Low represent an acceptable level of exposure and thus the site cannot be classified as impacted, regardless of the site linkage results.

When Chemical Exposure is Moderate or greater, the site linkage result indicates the likelihood that this exposure results from sediment contamination within the site. There is greater confidence that sediment contamination is the dominant source of tissue contamination at sites with Moderate or High Site Linkage and so these sites are classified as Likely or Clearly Impacted (fail HHSQO). The role of site sediment contamination is less certain for sites with Low site linkage, so the final assessment outcome is contingent on the severity of chemical exposure. Sites classified as having very low linkage do not fail the HHSQO for that contaminant group because contamination of the resident fish cannot be confidently associated with sediment contamination at the site; other sources of contamination are assumed to be more important in such cases.

Table 12.7. Tier 2 site assessment logic table.

Site Linkage	Chemical Exposure				
	Very Low	**Low**	**Moderate**	**High**	**Very High**
Very Low	Unimpacted	Unimpacted	Likely Unimpacted	Likely Unimpacted	Likely Unimpacted
Low	Unimpacted	Unimpacted	Likely Unimpacted	Possibly Impacted	Likely Impacted
Moderate	Unimpacted	Likely Unimpacted	Likely Impacted	Likely Impacted	Clearly Impacted
High	Unimpacted	Likely Unimpacted	Likely Impacted	Clearly Impacted	Clearly Impacted

Tier 2 Site Assessment Steps

Analyzing data and interpreting the results includes seven steps:

Step 1: Develop a conceptual site model

The conceptual site model is needed to define the site boundaries, guide selection of fish species to evaluate, develop the sampling design and schedule.

Step 2: Compile, review, and summarize data

The analytical chemistry results for sediment, water, and fish tissue are reviewed and validated according to the study's quality assurance plan. Concentration sums by contaminant class are calculated and the site mean, and standard error are calculated for total DDTs, total PCBs, total Chlordanes, and dieldrin. The dissolved water concentration of individual compounds (e.g., PCB congeners) is compiled and summarized to produce mean values.

Step 3: Input data into assessment tools

Enter the chemistry and supporting data into the DST worksheets or upload data files to the online assessment tool. Data for all chemical classes are entered and analyzed at the same time.

Step 4: Run the bioaccumulation model

Use the bioaccumulation models in the data analysis tools to calculate bioaccumulation factors for use in site linkage calculations (automated function in analysis tools).

Step 5: Use Monte Carlo Simulations to generate site linkage distributions and determine Site Linkage category

The linkage distribution plots are compared to the threshold categories to determine the Site Linkage category for each chemical class (automated function in analysis tools).

Step 6: Determine the Chemical Exposure category

Calculate weighted average fish tissue contaminant concentration based on the chemistry data and relative portion of diet for each species (automated function in analysis tools).

Step 7: Use the Chemical Exposure and Site Linkage results to determine the site assessment category

The indicator categories resulting from Steps 5 and 6 are compared to the logic table to determine the Tier 2 site assessment category for each contaminant class (automated function in analysis tools).

Unimpacted or Likely Unimpacted: site is not impacted with respect to HHSQO.

Possibly, Likely, or Clearly Impacted: sediment contamination at the site does not meet HHSQO.

Tier 2 Assessment Example using the DST

A sample dataset is provided to illustrate the Tier 2 assessment approach, and graphical depictions of the output. In this example, DDT, and PCB concentrations in fish, water and sediment are evaluated and integrated to make a site assessment. This example uses the DST to conduct the data analyses.

Step 1: Develop conceptual site model.

The CSM identified three fish species for assessment: spotted sand bass, shiner perch, and topsmelt. Composites of each species were collected over two sampling events and averaged to provide the tissue chemistry data. The sediment and water column samples were collected by grab and passive sampling at a single station, with each value representing the average of two sampling events.

Step 2: Compile, review, and summarize data

The raw sediment and water chemistry data for individual congeners and contaminant group constituents are checked and organized to select those constituents required for the analysis (Table 12.6). Sediment and tissue data are summed to calculate totals for each sample. Means and standard errors are calculated for the tissue and sediment (Table 12.7). Chlordane and dieldrin were not measured in this study, so an assessment cannot be conducted for these compounds. Site specific data on fish consumption are not available, so an equal proportion of consumption was assumed (0.33) for each of the three species.

Step 3: Input data into assessment tool

The site-specific characteristics (mean or representative values, Table 12.8) are entered into the DST Input worksheet. Contaminant sums for sediment and fish tissue (Table 12.7) are also entered on the DST Input worksheet. Individual compound concentrations for sediment and water are entered in the DST Contaminant Specific worksheet. Be sure to convert data to the correct units prior to entry the DST. The worksheet cells should be left blank if data are not available for a specific compound. Enter estimated values for nondetect constituents (do not enter special characters in cells, such as “<”).

Step 4: Run the bioaccumulation model

Run the “BiotaContamCalcs” macro in the DST by typing Ctrl-R. This macro applies the bioaccumulation model individually to each constituent entered on the Contaminant Specific worksheet. This step calculates the estimated tissue concentration for each component of the food web (target fish species and prey items) and back calculates the BSAF for each species and contaminant group. Note that model calculations are automatically conducted for each of eight fish dietary guilds, regardless of whether each guild was sampled for the study. However, the model results (e.g., BSAF and estimated tissue concentration) for the nontarget species will not be correct due to lack of site-specific tissue lipid data for those species. BSAF and guild summary results are shown in rows 164-172 of the DST Assessment Summary worksheet (Table 12.9).

Table 12.6. Sediment and water analytical data used in the assessment example.

Contaminant	Sediment Concentration (ng/g dry wt)	Dissolved Water Concentration (pg/L)
op-DDD	0.210	1.28
op-DDE	0.143	0.26
op-DDT	0.698	1.08
pp-DDD	0.630	4.19
pp-DDE	0.672	8.02
pp-DDT	0.765	1.23
PCB 8	0.193	5.55
PCB 11		
PCB 18	0.419	4.52
PCB 27		
PCB 28	0.305	2.65
PCB 29		
PCB 31		
PCB 33		
PCB 37	0.483	4.13
PCB 44	0.472	2.10
PCB 49	0.424	5.00
PCB 52	0.442	5.09
PCB 56		
PCB 60		
PCB 64		
PCB 66	0.475	1.87
PCB 70	0.363	2.56
PCB 74	0.421	1.15
PCB 77	0.100	0.24
PCB 81	0.076	0.44
PCB 87	0.168	1.00
PCB 95		
PCB 97		
PCB 99	0.690	9.59
PCB 101	0.760	10.08

Table 12.6. (Continued).

Contaminant	Sediment Concentration (ng/g dry wt)	Dissolved Water Concentration (pg/L)
PCB 105	0.183	1.19
PCB 110	0.373	4.43
PCB 114	0.109	0.69
PCB 118	0.754	6.20
PCB 119	0.072	0.61
PCB 123	0.114	1.15
PCB 126	0.025	0.02
PCB 128	0.218	1.56
PCB 132		
PCB 137		
PCB 138	0.738	4.87
PCB 141		
PCB 146		
PCB 149	1.186	9.36
PCB 151	0.127	1.33
PCB 153	1.976	14.03
PCB 156	0.087	0.43
PCB 157	0.031	0.16
PCB 158	0.056	0.32
PCB 167	0.059	0.36
PCB 168		
PCB 169	0.012	0.01
PCB 170	0.200	0.95
PCB 174		
PCB 177	0.250	1.80
PCB 180	0.382	1.48
PCB 183	0.133	0.72
PCB 187	0.683	3.30
PCB 189	0.040	0.13
PCB 194	0.204	0.21
PCB 195		
PCB 198		
PCB 199		
PCB 200	0.051	0.01
PCB 201	0.221	0.49
PCB 203		
PCB 206	0.195	0.08
PCB 209	0.268	0.01

Table 12.7. Summary sediment and fish chemistry data used in the assessment example.

Tissue/Matrix	Analyte	Mean	Standard error
Spotted Sand Bass	DDTs (ng/g ww)	1.40	0.41
Shiner perch	DDTs (ng/g ww)	6.06	1.38
Topsmelt	DDTs (ng/g ww)	6.61	1.28
Spotted Sand Bass	PCBs (ng/g ww)	26.30	3.66
Shiner perch	PCBs (ng/g ww)	35.40	7.16
Topsmelt	PCBs (ng/g ww)	51.60	16.02
Spotted Sand Bass	Lipid (%)	0.39	
Shiner perch	Lipid (%)	1.57	
Topsmelt	Lipid (%)	0.93	
Sediment	DDTs (ng/g dw)	0.67	0.02
Sediment	PCBs (ng/g dw)	10.08	0.39
Sediment	TOC (%)	1.51	

Table 12.8. Site-specific parameters for the assessment example.

Parameter	Mean
Area of Site (SA) (km^2)	1.26
Length of site (SL) (km)	1.98
Sediment Total Organic Carbon (TOC) (%)	1.51%
Dissolved Organic Carbon Content of water (DOCw) (kg/L)	1.27E-06
Particulate Organic Carbon Content of water (POCw) (kg/L)	1.57E-06
Mean Water Temp (T) (Deg. C)	21.0
Salinity (Sal) (PSU)	34.5
Dissolved Oxygen Concentration (DO) (mg/L)	7.8
Suspended solid concentration in water column (SSC) (kg/L)	2.46E-05

Table 12.9. Summary results produced after running DST "BiotaContamCalcs" macro. Note that concentration values refer to tissue and are expressed on a weight weight basis.

Species	Guild	DDTs BSAF (calc)	PCBs BSAF (calc)	DDTs Conc (ng/g)	PCBs Conc (ng/g)
Spotted Sand Bass	2 Benthic diet with piscivory	20.3	25.5	63.3	370.1
Shiner Perch	5 Benthic and pelagic without piscivory	7.9	10.1	24.6	146.6
Topsmelt	7 Benthic and pelagic with herbivory	3.4	4.3	10.6	62.6

Step 5: Use Monte Carlo Simulations to generate site linkage distributions and determine Site Linkage category

Monte Carlo Simulation is employed to obtain cumulative probability distributions for site linkage. This simulation uses the YASAIw add-in Monte Carlo Simulation macro for Excel (Eckstein and Riedmueller 2002, Pelletier 2009), which the user installs prior to using the DST. In the typical application, 10,000 simulations are performed.

To perform the simulation, click *Add-ins* (*Tools* in Excel versions before 2007) → *YASAI simulation*. A simulation menu box should appear. In the simulation box, change Sample Size to 10,000. All other options may be kept as defaults. Click the Simulate button, which will begin the MCS. Note that the sensitivity analysis option does not function in this version. This simulation will take a couple minutes to complete. After the MCS is completed, three new worksheets are produced: Simulation Output X, CFD Output X, and Iterations Output X. These worksheets are numbered sequentially and a new set of three will be added to the workbook each time the MCS is run (i.e., X=1, 2, 3….X). The results of the most recent MCS are extracted automatically and used to determine the site linkage category, which is shown on the Output Summary worksheet (Table 12.10). A plot of the site linkage distribution can be produced by entering the name of the current CFD Output worksheet into the Site Linkage Plot worksheet of the DST. The 25th percentile of both the DDT and PCB linkage distributions exceed the linkage threshold of 0.5 and are classified as having High Linkage.

Table 12.10. Summary of assessment results for data analysis example. Output generated using DST version 11.4.

Compound	Indicator	25th Percentile	50% Percentile or Mean	75th Percentile	95th Percentile	Category Score	Category
DDTs	Mean Weighted Observed Tissue Conc. (ng/g)		4.64				
	Mean Weighted Estimated Tissue Conc. (ng/g)		66.06				
	Chemical Exposure					1	Very Low
	Site Linkage	0.946	1.306	1.839		4	High
	Site Assessment					1	Unimpacted
PCBs	Mean Weighted Observed Tissue Conc. (ng/g)		37.39				
	Mean Weighted Estimated Tissue Conc. (ng/g)		118.86				
	Chemical Exposure					3	Moderate
	Site Linkage	2.295	3.179	4.516		4	High
	Site Assessment					4	Likely Impacted

Step 6. Determine the Chemical Exposure category

The categorical results for each indicator are obtained by comparing the weighted observed average tissue concentration to the threshold values (Table 10.2). Classification of Chemical Exposure is produced automatically by the DST and the results are listed in the Output Summary worksheet (Table 12.10). Tissue DDT concentration is below the OEHHA Fish Contaminant Goal (FCG) and is classified as Very Low for Chemical Exposure. Tissue PCB concentration falls within the range for ATL2 and is classified as Moderate for Chemical Exposure.

Step 7. Use the Chemical Exposure and Site Linkage results to determine the site assessment category

The categorical scores for the chemical exposure and site linkage categories are automatically integrated to obtain the final site assessment category. The results are included on the Output Summary worksheet (Table 12.10). The site is classified as Unimpacted for DDTs because Chemical Exposure is Very Low. The site is classified as Likely Impacted for PCBs due to the combination of unacceptable Chemical Exposure (Moderate) and High Site Linkage.

Tier 2 Assessment Example using the Online Tool

HHSQO Tier 2 assessment using the online tool follows the same general steps as for the DST. This tool is available at https://sccwrp.shinyapps.io/sqo_web/ and contains instructions for data entry and interpretation. Key features of this assessment tool are:

- An internet connection is required to use the tool.
- Data should be uploaded in the form of several files using .csv format:
  - **biota.csv** specifies the tissue lipid for each fish species,
  - **constants.csv** contains site-specific information, such as area and TOC,
  - **contam.csv** lists concentrations for sediment and water constituents,
  - **mcsparms.csv** contains tissue and sediment summary data needed for MCS analysis
- Templates for the data files can be downloaded from the web site. Please note that these files contain sample data that may need to be updated using site-specific results prior to use. Units for data are the same as for the DST, with two exceptions: values for tissue lipid and sediment TOC must be entered as kg/kg instead of %.
- Do not change the heading names or any other text in the data input files. Only numeric values should be changed.
- Data entry notes
  - **biota.csv:** The tissue lipid content of each of up to nine indicator species should be entered as kg/kg wet weight, which may need conversion from commonly reported percent format (e.g., 2% lipid = 0.02 kg/kg). Fish species data are

organized by indicator number (e.g., indic1, indic2, etc), which corresponds to a specific feeding guild type. **A lipid value must be entered for each indicator species, regardless of whether the study has data for that indicator.** The data file template contains default lipid values for use with species not included in your dataset: Required indicator species list for regulatory application: indic1=California halibut; indic2=spotted sand bass; indic3=queenfish; indic4=white croaker; indic5=shiner perch; indic6=common carp; indic7=topsmelt; indic8=striped mullet; indic9=white catfish.

- **constants.csv:** A numeric value must be provided for each data type in the file. **If a site-specific value is not available, then a default value, such as that included in the data file template, should be used.** Measurement units must be the same as those specified in the csv file notes.

- **contam.csv:** This file lists concentrations for the individual component of each of four chemical groups: chlordanes, dieldrin, DDTs, and PCBs. Average sediment and dissolved water column concentrations representative of the site are required and should be entered into columns cs_ng.g and cd_ng.g, respectively. **If data are not available for some components (e.g., specific PCB congeners), then leave the cell blank.** The tool will estimate dissolved chemical concentrations for the water column and pore water if the data are not present. Units must be ng/g dry weight for sediment and pg/L for water.

- **mcsparms.csv:** This file contains three types of data needed to perform the MCS routine: proportion of the seafood diet represented by each fish type (propseaf), mean and SE of tissue concentration for each chemical group by fish guild number, and mean and SE of sediment concentration for each chemical group. Leave the cell blank if data are not available.

  The "propseaf" value indicates the proportion (ranging from 0 to 1) of the human diet represented by a given indicator species. **Note that a propseaf value is required for all nine indicator species and they must sum to exactly 1 for the analysis to run correctly.** A default assumption of approximately equal proportions should be used unless site-specific data is available. For example, using three indicators: indic1propseaf=0.33; indic3propseaf=0.33; indic5propseaf=0.34; propseaf for all other species = 0.

  Tissue contaminant data must be entered in units of ng/g wet wt. Sediment contaminant data must be entered in units of ng/g dry wt. **Be sure to enter the SE and not the standard deviation of the concentration.**

Tier 3

Tier 3 represents an alternative assessment of the HHSQO with greater site specificity and more flexibility than Tier 2. This option has been established to address stakeholder concerns that the standardized Tier 2 assessment may not have sufficient sophistication, resolution, or site specificity to accurately evaluate site linkage or potential human health impacts from sediment contamination. More complex models and data analyses are also useful for developing

contaminated sediment management or remediation plans (e.g., identify sites for dredging, forecasting changes in sediment quality over time). However, a lower level of complexity may be sufficient for HHSQO assessment, where the objective is primarily to determine whether unacceptable human health impacts are associated with current site sediment contamination conditions.

A Tier 3 assessment can take many forms, such as use of different bioaccumulation models/parameters for the site linkage calculation, use of nonstandard seafood species, different thresholds to assess chemical exposure, consideration of other sources of chemical exposure, or consideration of spatial or temporal variability in contamination. The potential benefits of a Tier 3 assessment are countered by several disadvantages. Tier 3 assessment is likely to be more expensive, more time-consuming, and yield results that may not be comparable to assessments based on Tier 2 methods or from other studies. Thus, the decision to conduct a Tier 3 assessment should be made with the approval of the regulatory agency and be based on evidence that conditions (trigger criteria) exist that indicate a potential for more accurate or useful assessment results.

Tier 3 Objectives

A Tier 3 assessment may be performed to address unique situations or evaluate factors affecting the assessment not considered in Tier 2. The objective of Tier 3 assessment might include:

- Improve accuracy and precision of the assessment
- Evaluate different risk related assumptions associated with chemical exposure determination
- Incorporate spatial and temporal factors into the assessment
- Evaluate specific sub-areas, contaminant gradients or potential hotspots

Tier 3 Trigger Criteria

Before deciding to proceed with a Tier 3 assessment, there should be evidence indicating that the Tier 2 assessment outcome is likely incorrect (e.g., incorrect classification of chemical exposure or site linkage). In general, the site should meet one of the following conditions (trigger criteria):

1. Variation in factors or processes are present that affect contaminant bioaccumulation from sediment, potentially resulting in a difference in site linkage category. Examples include:
    - Differences in the relationship between geochemical characteristics and contaminant bioavailability
    - Differences in physiological processes affecting bioaccumulation model performance, such as growth rate or assimilation efficiency
    - Measured sediment concentrations are not representative of actual fish forage area due to spatial or temporal variations in sediment contaminant distribution, fate, or transport

2. Differences in food web or forage range of target species

    - Use of sport fish species other than those listed in Table 10.1

    - Regional differences in fish diet

3. Changes in exposure factors that are likely to result in a difference in chemical exposure category. Examples include:
    - Consumption rate
    - Proportion of each sport fish species consumed by humans

4. Presence of spatial or temporal factors likely to affect classification of site linkage. Examples include:

    - Sediment contamination hot spots
    - Temporal change in loading rates
    - Substantial offsite sediment contamination

Tier 3 Assessment Considerations

The Tier 3 approach is site-specific and should be developed based on a conceptual site model, in addition to considerations identified by stakeholders. If a Tier 3 analysis is employed, the specific modifications to the approach should be determined by the information needs for the site in question.

Tier 3 assessments can include a bioaccumulation modeling approach different from that included in the Tier 2 Decision Support Tool. This could include mechanistic models of contaminant fate and transport, in addition to the movement of individual fish. USEPA (2009) provides recommendations regarding how to select a modeling approach in a Tier 3 type of assessment. The processes included and model complexity should be chosen based on the assessment questions, data availability, and available resources (USEPA 2009).

Tier 3 assessments could incorporate more sophisticated treatments of uncertainty and variability. This may include sensitivity analyses or uncertainty analyses of the calculations of chemical exposure and site linkage. Such analyses could identify local sources of uncertainty, as well as potentially incorrect model assumptions.

Additional data collection may be incorporated into Tier 3 analyses. Examples of local data collection that may be warranted include:

- Seafood consumption surveys to determine local consumption rates

- Development of local parameter values for food web structure and diets of indicator fish species, to parameterize bioaccumulation models and better characterize site linkage

- Measurement of porewater contaminant concentrations to improve estimates of the contribution of this compartment to bioaccumulation.

- Contaminant monitoring of offsite sediment, as well as concentrations in other possible sources (e.g., stormwater or creek discharge during runoff events).
- Detailed monitoring of site sediment contamination to identify potential “hotspot” areas of elevated seafood exposure, and to better characterize the spatial extent of contamination
- Seasonal monitoring of indicator fish species, to determine seasonal variation in chemical exposure to seafood consumers

Tier 3 Assessment Steps

The specific process for conducting a Tier 3 assessment will vary among applications because of variability in the data analysis methods. In general, the process is expected to be similar to that described for Tier 2.

Interpretation of Tier 3 Assessment Results

Interpretation of the results of the Tier 3 assessment should be based on the same indicators, thresholds, and integration logic described for Tier 2. For example, even though a different type of bioaccumulation model might be used in the assessment, site linkage should still be scored as either Very Low, Low, Moderate, or High based on a probability distribution of linkage values. Use of the same indicators and integration relationships will help maintain comparability between Tier 3 and Tier 2 assessments.

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Appendix A: Using the RIVPACS Calculation Tool on the SCCWRP Website

This tool was developed to provide an easy way for people to upload their benthic data and have the RIVPACS Benthic Index calculated for each of their sites. The tool uses an Excel based template to submit data for analysis. The calculations are done using script in the R programing language.

The RIVPACS index calculates the number of reference taxa present in the test sample (observed or "O") and compares it to the number expected to be present ("E") in a reference sample from the same habitat.

Here are the steps necessary to successfully upload your data:

1. Download the RIVPACS excel template from the RIVPACS Calculator Tool website.

2. Fill in columns A through E on the StationInfo tab. This includes StationID, Sample Depth (must be in meters), Latitude (decimal degrees), Longitude (decimal degrees and must be negative) and the HabitatCode ("C" for Southern California Marine Bays and Estuaries and "D" for San Francisco Polyhaline).

3. Fill in columns A through C on the Benthic tab with StationID, taxa, and abundance. It is IMPORTANT not to move (cut and paste) lines of data once they have been entered into the sheet. Names of the tabs within the input file must not be changed.

4. Check your data using Column D. When you fill in Column B a check is done against the Southern California Association of Marine Invertebrate Taxonomists (SCAMIT) Edition 5 list (TaxaList tab in the Excel workbook).

    a. Column D is used to check your species against the SCAMIT Edition 5 List (TaxaList tab in excel workbook). If your taxon name matches a taxon on this list, you will see the word "Correct" in column D. If you see the word "Wrong" in Column D, your taxon does not match any on the SCAMIT list. This may be due to a misspelling in your data or a difference in taxonomic nomenclature or identification. Spelling is critical, including punctuation (e.g., no periods after "sp") and abbreviations. The TaxaList page can be consulted for help with proper spelling.

    b. Column D has formulas for the checks down to row 30000. If you have data that goes beyond row 30000, please copy and paste the formula down to the end of your data. We do this to limit the size of the template for downloading.

2. Once you have filled in the template, you are ready to submit your data. From the RIVPACS Calculator Tool Website you can click on the Browse button, choose your file, and click on Open. In addition, you can also drag and drop your file anywhere on the web page (Note: this feature does not work if you are using Internet Explorer). Your file will be automatically processed.

3. Once your file is submitted, the results will appear automatically in a series of links on the page, organized by habitat (southern California and San Francisco Bay). You can click on the links to see specific RIVPACS output. The output pages are in HTML format which can be saved. The saved HTML files can then be opened in Excel or Word for data manipulation and use in other index or assessment tools.

    a. The RIVPACS Output page has O/E data that is the key result. This page contains the calculated number of observed reference species ("O"), expected species ("E"), and then the ratio ("O/E"). This page also shows the results of outlier checking which determines whether the stations belong in their designated habitat based on latitude, longitude and depth.

    b. The Probability Comparison link contains up to three tables depending on the combination of station location and depth and taxa present. One table may include the taxa observed but not predicted. Another table may include the taxa predicted but not observed. The final table may contain the taxa predicted that were observed. Along with the taxa each table also shows at which station the information pertains along with the probability of the taxa being found.

    c. The Probability Matrix shows the predicted occurrence probability for each of the reference taxa used in the RIVPACS calculation. You can submit multiple files for calculation and a new set of links will appear for each submittal.

APPENDIX B: USING THE STATISTICAL ANALYSIS SYSTEM (SAS) FOR RIVPACS CALCULATIONS

Overview

Two user-created files containing (1) habitat and (2) macrofauna data are submitted to a SAS program together with three provided data files containing (1) reference sample habitat data, (2) reference group species occurrence expectations, and (3) a master species list (Table B1). The SAS Program checks the samples in the test macrofauna data for presence in the habitat data, and taxon names against the master species list. Then it calculates RIVPACS O/E values and condition categories for each sample. Separate program and data files are provided for evaluating data from Southern California Marine Bays and San Francisco Bay Polyhaline.

Methods

Running RIVPACS analysis using the Statistical Analysis System (SAS) computer program provided on the SCCWRP web site is a three-step process.

1. Prepare data files.
2. Specify folder and file names.
3. Run the computer program.

Prepare data files

The habitat data and macrofauna data for the test samples are prepared by the user as SAS data files, while the other necessary files (Table B1) are available on the SCCWRP web site. The user prepared files must contain one or more sample identifier variables; these variables are specified by the user. Examples of user prepared data files are provided on the SCCWRP web site. The example files use a single variable named “Site” to uniquely identify each sample.

In addition to identifier variables, habitat data files for Southern California Marine Bays contain Sample Depth (in meters) as well as Latitude and Longitude in decimal degrees (Table B2). San Francisco Bay Polyhaline habitat data files contain a Hab_G variable instead of latitude (Table B3). The Hab_G variable represents a dummy variable used in model development and is zero for samples from San Francisco Bay Polyhaline. In all user created files, the data variable names and characteristics must exactly follow tables B2 to B4. The number of sample identifier variables and their names and characteristics are entirely at the user’s discretion, but they must match exactly between the habitat and macrofauna data files.

Table B1. Computer files for calculating RIVPACS using SAS.

Contents	Source	File Name	
		Southern California Marine Bays	San Francisco Bay Polyhaline
SAS Program	Provided	RIVPACS_SouthernCaliforniaMarineBays.sas	RIVPACS_PolyhalineCentralSanFranciscoBay.sas
Master Species List	Provided	SasMasterBenthicTaxonListAll_20071231.sas7bdat	
Reference Habitat Data	Provided	SasSoCalMB_RefHabitat.sas7bdat	SasPCSFB_RefHabitat.sas7bdat
Reference Species List	Provided	SasSoCalMB_RefSpecies.sas7bdat	SasPCSFB_RefSpecies.sas7bdat
Reference Species Expectations	Provided	SasSoCalMB_RefSpeciesExpect.sas7bdat	SasPCSFB_RefSpeciesExpect.sas7bdat
Test Sample Habitat Data	User Created	SasSoCalMB_TestHabitatExample.sas7bdat	SasPCSFB_TestHabitatExample.sas7bdat
Test Sample Macrofauna Data	User Created	SasSoCalMB_TestFaunaExample.sas7bdat	SasPCSFB_TestFaunaExample.sas7bdat

Table B2. Contents of user created habitat data files for Southern California Marine Bays.

Variable	Type	Length	Format	Units
Latitude	Numeric	8 bytes	11.6	Decimal degrees
Longitude	Numeric	8 bytes	11.6	Decimal degrees
SampleDepth	Numeric	8 bytes	4.1	Meters
Sample Identifier 1	User's choice, but consistent with macrofauna data			
Sample Identifier 2	User's choice, but consistent with macrofauna data			
Sample Identifier n	User's choice, but consistent with macrofauna data			

Table B3. Contents of user created habitat data files for San Francisco Bay Polyhaline.

Variable	Type	Length	Format	Units
Hab_G	Numeric	8 bytes	1.0	None
Longitude	Numeric	8 bytes	11.6	Decimal degrees
SampleDepth	Numeric	8 bytes	4.1	Meters
Sample Identifier 1	User's choice, but consistent with macrofauna data			
Sample Identifier 2	User's choice, but consistent with macrofauna data			
Sample Identifier n	User's choice, but consistent with macrofauna data			

For both habitats, the test macrofauna data is a simple file with taxon names and abundances on each row in addition to the identifier variable(s). Each row contains the name and abundance for one taxon (Table B4).

Table B4. Contents of user created macrofauna data files for Southern California Marine Bays and San Francisco Bay Polyhaline.

Variable	Type	Length
Abundance	Numeric	8 bytes
TaxonName	Character	50
Sample Identifier 1	User's choice, but consistent with habitat data	
Sample Identifier 2	User's choice, but consistent with habitat data	
Sample Identifier n	User's choice, but consistent with habitat data	

Specify folder, identifier variable, and file names

Edit the five lines that immediately follow the instructions at the beginning of the program file:

- Specify the folder where the data are stored in the Libname statement.
- Specify the names of the variables in your data that uniquely identify each sample in the "identifier" instruction. The example data contain a single identifier variable named "Site."
- Specify the names of the files containing habitat and macrofauna data to be analyzed, and the name of a file to be created to store results.

Run the computer program

Submit the data to run in SAS. The program output includes the identifier variables, RIVPACS O, E, and O/E values, and condition categories for each sample. Two types of output are generated: an output window and a stored results file. The output window contains the analysis results and describes the contents of the SAS data file that is created to store them. The stored results file can be used to facilitate data transfer or subsequent data analysis.

APPENDIX C: SORTING AND RE-ANALYSIS FORMS

Sorting Form:

Station: ________ Analytical Laboratory: ________

Sorted by: ________ Sorting Laboratory: ________

Date Sorting Begins: __/__/____ (mm/dd/yyyy) Total time (hours):

# of Taxa Lots in Sample: ________ # of Sample Containers ________

Comments: ________________________________

Quality Control Re-Sort

Re-sorted by: ________ Date of re-sort: __/__/____ (mm/dd/yyyy)

Percent Sorting Efficiency = {**A** / [**A** + (**B**/**C**)]}*100

A = # of Organisms originally sorted: ________
B = # of Organisms found in resort: ________
C = Fraction of sample re-sorted (i.e., aliquot): ________
Note: C = 1 if entire sample re-sorted
% Sorting Efficiency = ________

Quality Control Actions: ________________________________

____

Note: no action needed if sorting efficiency ≥ 95%

Signed: ________________
Responsible Supervisor

Discrepancy Classifications and Resolution Codes

Discrepancy Classifications:

E = Error (identification or count)

J = Judgmental difference (difference level of expertise)

N = Nomenclatural difference (naming convention usage)

L = Apparent specimen loss (sample handling)

P = Processing error (data entry, animal from another vial)

Resolution codes:	Error type (* requires data change)	Action
1 = Primary taxonomist misidentification	True*	Training
2 = QC taxonomist misidentification	True	Training
3 = Primary taxonomist miscount	True*	Review best practices
4 = QC taxonomist miscount	True	Review best practices
5 = Primary taxonomist data entry error	Random*	Review best practices
6 = QC taxonomist data entry error	Random	Review best practices
7 = Primary naming convention discrepancy	True*	Review best practices
8 = QC naming convention discrepancy	True	Review best practices
9 = Primary variation in level of expertise	Non Error	Training
10 = QC variation in level of expertise	Non Error	Training
11 = organism added from another vial[+] (vials other than Annelid fragments and Ophiuroid arms, in which case those would be considered misidentification errors)	Random*	Review best practices
12 = organism lost	Random	Review best practices
13 = specimen vouchered	Non-Error	Data Tracking
14 = specimen damaged during primary ID, not identifiable by QC taxonomist	Non-Error	No Action

Discrepancy Report:

SQO Example

MACROFAUNAL ANALYSIS QC

Date ____________

DISCREPANCY REPORT

Station ____________________

Analytical Laboratory (Lab A): ____________________ Date Analyzed: ____________

Re-Analytical Laboratory (Lab B): ________________ Date Re-Analyzed: __________

Line #	Re-Identification	Re-Count	Orig. Count	Original Identification	Comments
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					

Discrepancy Report (cont'd)

Date ______________

Station ____________________ Page 2 of ______

Line #	Re-Identification	Re-Count	Orig. Count	Original Identification	Comments
27					
28					
29					
30					
31					
32					
33					
34					
35					
36					
37					
38					
39					
40					
41					
42					
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49					
50					
51					
52					
53					
54					
55					
56					
57					
58					
59					

Discrepancy Report (cont'd)

Date ____________________

Station ____________________ **Page _____ of _____**

Line #	Re-Identification	Re-Count	Orig. Count	Original Identification	Comments

Discrepancy Resolution Report:

SQO Example
MACROFAUNAL ANALYSIS QC

Date ______________________

DISCREPANCY REPORT

Page 1 of ______

Station ______________________

Analytical Laboratory (Lab A): ______________________ Date Analyzed: _____________

Re-Analytical Laboratory (Lab B): __________________ Date Re-Analyzed: ___________

Line #	Re-Identification	Re-Count	Orig. Count	Original Identification	Comments
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					

Discrepancy Resolution Report (Cont'd)

Date________

Station ____________________ **Page** ____ **of** ____

Line # refers to Discrepancy Report (a discrepancy may involve multiple line #s)

		Do not write in these columns			
				Effect of Resolution on Orig. Results	
Line #	**COMMENTARY** *Provide resolution and explanation of all discrepancies (use as many lines as necessary for each discrepancy)*	**Discep. Class.**	**Resol. Code**	**SPP # (+/-)**	**Count (+/-)**

Discrepancy Classification: E = Error J = Judgmental Diff. N = Nomenclature L = Apparent Specimen Loss

Resolution Code: 1 = Lab A misID 2 = Lab B misID 3 = Lab A mis Count 4 = Lab B mis Count

Infaunal Identification and Enumeration Accuracy Report

SQO Example
MACROFAUNA ID & ENUMERATION — ACCURACY REPORT — Date____________

Station ________________________

Analytical Laboratory (Lab A): __________________________ Date Analyzed:

Re-Analytical Laboratory (Lab B): _______________________ Date Re-Analyzed: _________

% ERROR OF ANALYSIS: Percent error is calculated for three aspects of sample analysis. Error in the # of taxa discriminated and total organism count may be either + or -. These provide measures of the data quality as relates to parameters such as species richness, abundance, and diversity. Identification accuracy is expressed as percent error in identification of individual taxa. It provides a measure of the quality of the data as a representation of community composition.

# of Taxa Discriminated:	Total Organism Count:	Identification Accuracy:
[(X-Y)/X] * 100	[(X-Y)/X] * 100	[X/Y] * 100
where	*where*	*where*
X = # of Taxa after discrepancy resolution	*X = # in Resolved count*	*X = # of Taxa changed*
Y = # of Taxa originally discriminated	*Y = # in Original count*	*Y = # of Taxa after discrepancy resolution*
X = Y=	X = Y=	X = Y=
Score:________%	Score:________%	Score:________%

COMMENTS ____

APPENDIX D: BENTHIC HABITAT TYPES

Six assemblage types were identified in California bays and estuaries by Ranasinghe *et al.* (2012). The assemblage types correspond to physical habitat differences, which result in benthic species composition differences that can be used to verify habitat type membership.

The six habitat types were:

Southern California Marine Bays (Habitat C)

San Francisco Bay Polyhaline (Habitat D)

Shallow Wetlands and Coastal Bays (Habitat E)

Very Coarse Saline Sediments (Habitat F)

San Francisco Bay Mesohaline (Habitat G)

Oligohaline and limnetic waters (Habitat H).

The habitat type is determined by physical habitat characteristics and can be verified by species composition. The physical habitat criteria for inclusion in these habitats are presented in Table D1. The criteria vary slightly in different latitudinal segments, reflecting differences in exposure to freshwater inputs due to climatic differences. Within San Francisco Bay, freshwater and sediment grain size influences were integrated by geography.

Table D1. Criteria for determining habitat type.

Latitudinal Segment	Criteria and Habitat

San Francisco Bay Latitude
(37.4˚ to 38.25˚N)
	Fines ≤ 2.5%: **Habitat F**
	Otherwise
	    Inside San Francisco Bay:
	        ▪ Red Rock Location: **Habitat F**
	        ▪ Shallow wetland samples: **Habitat E**
	        ▪ Otherwise
	            • Dumbarton Bridge to Richmond-San Rafael Bridge: **Habitat D**
	            • N of Richmond-San Rafael Bridge or S of Dumbarton Bridge: **Habitat G**
	    Outside San Francisco Bay: **Habitat E**

Central California
Latitude
(34.5˚ to 37.4˚N)
	Salinity < 7.5 psu: **Habitat H**
	Otherwise
	    Fines ≤ 2.5%: **Habitat F**
	    Otherwise: **Habitat E**

Southern California
Latitude
(< 34.5˚N)
	Salinity < 10 psu: **Habitat H**
	Otherwise
	    Fines ≤ 2.5%: **Habitat F**
	    Otherwise
	        Salinity 10-27 psu: **Habitat E**
	        Salinity > 27 psu: **Habitat C**

Northern California and
Oregon
Latitude
(38.25˚ to 47.0˚N)
	Salinity < 5 psu: **Habitat H**
	Otherwise
	    Fines ≤ 2.5%: **Habitat F**
	    Otherwise: **Habitat E**

Puget Sound Latitude
Latitude
(> 47.0˚N)
	Salinity < 5 psu: **Habitat H**
	Otherwise
	    Fines ≤ 2.5%: **Habitat F**
	    Otherwise
	        ▪ Coastal Bays (Longitude > 123.8˚W): **Habitat E**
	        ▪ Fines < 20: **Habitat B**
	        ▪ Fines ≥ 41%: **Habitat A**
	        ▪ Fines ≥ 20-<41%
	            • Depth < 75m: **Habitat B**
	            • Depth ≥ 75m: **Habitat A**

Table D2. Species characteristic of west coast bay and estuary habitats.
Presented are exclusivity values for abundant (mean abundance > 100 m^{-2}) taxa with fidelity > 50% or exclusivity > 80% in each assemblage. Taxonomic nomenclature for provisional taxa (e.g., *Cossura* sp A) follows SCAMIT Edition 4 (Southern California Association of Marine Invertebrate Taxonomists 2001). Fidelity was calculated as the frequency of occurrence of a taxon in assemblage samples, expressed as a percentage. Exclusivity was the abundance of a taxon in assemblage samples, expressed as a percentage of its total abundance in all samples.

Taxon	Higher Taxon	Assemblage							
		A	B	C	D	E	F	G	H
Euphilomedes product	Arthropoda : Ostracoda	92							
Eudorella pacifica	Arthropoda : Cumacea	91							
Axinopsida serricata	Mollusca : Bivalvia	89							
Protomedeia articulata Complex	Arthropoda : Amphipoda	89							
Protomedeia grandimana	Arthropoda : Amphipoda	82							
Amphiodia spp.	Echinodermata : Ophiuroidea	73							
Prionospio (Minuspio) lighti	Annelida : Polychaeta	68							
Levinsenia gracilis	Annelida : Polychaeta	47							
Ericthonius rubricornis	Arthropoda : Amphipoda		100						
Phyllochaetopterus prolific	Annelida : Polychaeta		100						
Ampelisca agassizi	Arthropoda : Amphipoda		100						
Alvania compacta	Mollusca : Gastropoda		94						
Tellina modesta	Mollusca : Bivalvia		89						
Rochefortia tumida	Mollusca : Bivalvia		84						
Aphelochaeta glandaria Complex	Annelida : Polychaeta		81						
Prionospio (Prionospio) dubia	Annelida : Polychaeta		71						
Nutricola lordi	Mollusca : Bivalvia		63						
Parvilucina tenuisculpta	Mollusca : Bivalvia		55						
Euphilomedes carcharodonta	Arthropoda : Ostracoda		49	15					
Mediomastus spp.	Annelida : Polychaeta		9	42	16				
Amphideutopus oculatus	Arthropoda : Amphipoda			100					
Caecum californicum	Mollusca : Gastropoda			100					
Cossura sp A	Annelida : Polychaeta			100					
Barleeia spp.	Mollusca : Gastropoda			100					
Synaptotanais notabilis	Arthropoda : Tanaidacea			100					
Scoletoma sp C	Annelida : Polychaeta			100					
Paracerceis sculpta	Arthropoda : Isopoda			99					
Prionospio (Prionospio) heterobranchia	Annelida : Polychaeta			99					
Fabricinuda limnicola	Annelida : Polychaeta			99					
Tagelus subteres	Mollusca : Bivalvia			96					
Pseudopolydora paucibranchiata	Annelida : Polychaeta			89					
Musculista senhousia	Mollusca : Bivalvia			87					
Theora lubrica	Mollusca : Bivalvia			72					
Pista percyi	Annelida : Polychaeta			65					
Leitoscoloplos pugettensis	Annelida : Polychaeta			63					
Euchone limnicola	Annelida : Polychaeta			45					
Exogone lourei	Annelida : Polychaeta			28	56				
Crepidula convexa	Mollusca : Gastropoda				100				

Table D2. (Continued).

Taxon	Higher Taxon	Assemblage							
		A	B	C	D	E	F	G	H
Sabaco elongates	Annelida : Polychaeta				99				
Ampelisca abdita	Arthropoda : Amphipoda				94				
Caprella spp.	Arthropoda : Amphipoda				94				
Sinocorophium heteroceratum	Arthropoda : Amphipoda				94				
Molgula spp.	Chordata : Ascidiacea				92				
Photis brevipes	Arthropoda : Amphipoda				90				
Sphaerosyllis californiensis	Annelida : Polychaeta				87				
Monocorophium acherusicum	Arthropoda : Amphipoda				84				
Leptochelia dubia	Arthropoda : Tanaidacea				72				
Oligochaeta	Annelida : Oligochaeta				8	60			19
Americorophium stimpsoni	Arthropoda : Amphipoda					100			
Pygospio elegans	Annelida : Polychaeta					99			
Eogammarus confervicolus Complex	Arthropoda : Amphipoda					99			
Americorophium spinicorne	Arthropoda : Amphipoda					98			
Hobsonia florida	Annelida : Polychaeta					97			
Gnorimosphaeroma insulare	Arthropoda : Isopoda					97			
Potamopyrgus antipodarum	Mollusca : Gastropoda					93			
Cryptomya californica	Mollusca : Bivalvia					91			
Pseudopolydora kempi	Annelida : Polychaeta					91			
Neanthes limnicola	Annelida : Polychaeta					87			
Gnorimosphaeroma oregonense	Arthropoda : Isopoda					83			
Macoma balthica	Mollusca : Bivalvia					82			
Capitella capitata Complex	Annelida : Polychaeta					82			
Eohaustorius estuaries	Arthropoda : Amphipoda						90		
Corbula amurensis	Mollusca : Bivalvia							99	
Marenzelleria viridis	Annelida : Polychaeta							98	
Insecta	Arthropoda : Insecta								99
Boccardiella ligerica	Annelida : Polychaeta								93
Corbicula fluminea	Mollusca : Bivalvia								92
Chironomidae	Arthropoda : Chironomidae								86
Americorophium salmonis	Arthropoda : Amphipoda								29

APPENDIX E: ADJUSTING TAXONOMIC NOMENCLATURE

> "*'What's the use of their having names,' the Gnat said, 'if they won't answer to them?'*
> *'No use to them,' said Alice; 'but useful to the people who name them, I suppose. If not, why do things have names at all?'*"
> – Lewis Carrol, from The Complete Alice in Wonderland Adventures

Invertebrate zoology, taxonomy, and our understanding of how different species of organisms are related to each other is an evolving science. As such, the names that are used for different species will change from time to time to better reflect our understanding of the fauna. While this represents the best and most correct science, it creates some problems for benthic condition assessment tools like those are used as part of the SQO program.

The currency of the benthic condition tools that have been developed are the names and abundances of the taxa found in sample. As such, the calibration and validation of a given index is going to be associated with the taxonomic standards of the time when the index was developed. The RBI, IBI, BRI, and RIVPACS indices used in the calculation of SQO Benthic LOE were completed in 2008. As such, the names used in the tools are those listed in the SQO Species List (available at www.sccwrp.org), which in turn had been standardized to the SCAMIT species list edition 5.

Taxonomic standards have continued to evolve, and the names assigned to some taxa have changed since 2008 – including some of those taxa used in the calculation of the RBI, IBI, BRI, and RIVPACS. At present, it would be a considerable undertaking to recalibrate all these indices to a 2019 taxonomic standard. In lieu of fully re-calibrating all of the indices, the best option to maintain the efficacy and validity of the benthic indices and their associated condition thresholds is to modify the more modern taxonomic nomenclature so that it best matches the standard used during the creation of the indices.

Table E1 represents our best recommendations for rolling back 2019 standard taxonomy (i.e., SCAMIT Species List ed 12) to that of the SQO species list. Our recommendation is that if any of the taxa observed in a sample from the present-day match those in the 2019 Species Names column, they should be changed to the corresponding name in the SQO Species List Name column.

Table E1 represents a good tool for rolling back names from the 2019 standard to names compatible with the RBI, IBI, BRI, and RIVPACS indices. The M-AMBI calculator was developed to the taxonomic standard of 2019. However, it is reasonable to assume that as taxonomic nomenclature continues to evolve and improve, some names used in calculating any of these indices will further change in the future. To continue to use the benthic indices, future names will have to be rolled back to match the standard of the data used to calibrate the different indices. Our first recommendation is to include local taxonomic experts who work with benthic infauna in any potential changes. Secondly, we would recommend using species lists like the most recent SCAMIT species list at the time of sample collection or the World Register of Marine Species database (www.marinespecies.org) that catalog species synonyms (i.e., previously used names that are no longer valid for that taxon). With those two provisions, names could be rolled back using the following steps:

1. Match the synonyms of valid, present-day taxa to the taxa of the SQO Species List
2. Where there are matches, names could be changed using the following series of criteria
    a. Is the present-day name on the SQO Species List? If yes, do not change the name. If no, then
    b. Is the present-day name a one-to-one change with its synonym (e.g., Species A used to be named Species B)? If yes, change the present-day name to the synonym. If no, then
    c. Is there more than one present-day name associated with a single synonym (e.g., Species A and Species B both used to be named Species C)? If yes, change all of the present-day names to the synonym. If no, then
    d. Is there more than one present-day name associated with more than one synonym (e.g., Some of Species A and some of Species B used to be named Species C and others of Species A and others of Species B used to be named Species D)? If yes, consult with taxonomic expert for the species in question. Determine if all of the synonyms are recognized by the SQO indices (i.e., they have a BRI tolerance value or a RIVPACS value). If only one of the synonyms is recognized by the indices, consider, with the input of the taxonomic expert, changing all present-day names to that single synonym, while realizing this is not taxonomically ideal it may be practical for the purposes of the assessment.
3. If no synonyms of the present-day name match the SQO Species List, then keep the present-day name.

Table E1. Recommended name changes to be made to benthic infauna to roll back data created using a 2019 taxonomic standard to be most compatible to benthic indices that use SQO Species List names (e.g., RBI, IBI, BRI, and RIVPACS).

2019 Species Name	SQO Species List Name	Synonym Describer
Acanthinucella spirata	Acanthina spirata	of McLean 1978
Acromegalomma pigmentum	Megalomma pigmentum	Reish 1963
Alamprops carinatus	Lamprops carinatus	of SCAMIT Ed 11
Alamprops quadriplicatus	Lamprops quadriplicatus	of SCAMIT Ed 11
Alderia willowi	Alderia modesta	of authors SCB not (Lóven 1844)
Alitta succinea	Neanthes succinea	(Leuckart 1847)
Amage scutata	Paramage scutata	of Williams 1987
Annuloplatidia hornii	Platidia hornii	of SCAMIT Ed 10
Aphelochaeta sp A	Aphelochaeta glandaria Cmplx	of SCAMIT Ed 8 in part
Aphelochaeta sp LA1	Aphelochaeta petersenae	of Rowe 1996 º not Blake 1996
Astropecten californicus	Astropecten verrilli	of authors NEP not de Loriol 1899
Bipalponephtys cornuta	Nephtys cornuta	Berkeley & Berkeley 1945
Caesia perpinguis	Nassarius perpinguis	of McLean 1996
Californiconus californicus	Conus californicus	Hinds 1844
Callianax baetica	Olivella baetica	Carpenter 1857
Callianax biplicata	Olivella biplicata	of authors NEP
Campylaspis sp B	Campylaspis biplicata	of authors NEP in part not Watling & McCann 1997
Caprella californica Cmplx	Caprella californica	of authors NEP not Stimpson 1857 [in part]
Caprella mendax	Caprella equilibra	of Dougherty & Steinberg 1953 not Say 1818
Caryocorbula porcella	Corbula porcella	Dall 1916
Ciona robusta	Ciona intestinalis	of SCAMIT Ed 10 not Linnaeus 1767
Crangon alaskensis	Crangon nigricauda	of Holmes 1900 not Stimpson 1856
Crepipatella lingulata	Crepipatella dorsata	of SCAMIT Ed 4
Cyanoplax hartwegii	Lepidochitona hartwegii	of authors NEP
Decipifus penicillata	Nassarina penicillata	of McLean 1969
Deflexilodes similis	Deflexilodes norvegicus	of authors NEP not (Boeck 1861)
Dialychone albocincta	Chone albocincta	Banse 1972
Dialychone veleronis	Chone veleronis	Banse 1972
Drilonereis mexicana	Drilonereis nuda	of Hartman 1944 in part not Moore 1909
Edwardsia juliae	Edwardsia sp G	MEC 1992 º
Edwardsia olguini	Scolanthus sp A	SCAMIT 1983 º in part
Ericerodes hemphillii	Podochela hemphillii	of SCAMIT Ed 7
Eualus subtilis	Eualus lineatus	of authors NEP in part
Gadila aberrans	Cadulus aberrans	Whiteaves 1887
Garnotia adunca	Crepidula adunca	G. B. Sowerby I 1825
Glycera tesselata	Glycera nana	of Treadwell 1914 not Johnson 1901
Goniada brunnea	Goniada maculata	of Hartman 1940 not Ørsted 1843
Hemiproto sp A	Hemiproto sp A	Benedict 1978 º
Heptacarpus stimpsoni	Heptacarpus cristatus	of Holmes 1900
Hermundura fauveli	Parandalia fauveli	Berkeley & Berkeley 1941
Hesperato columbella	Erato columbella	Menke 1847

Table E1. (Continued).

2019 Species Name	SQO Species List Name	Synonym Describer
Heteromastus filiformis Cmplx	Heteromastus filiformis	of authors NEP not (ClaparΦde 1864)
Heterophoxus affinis	Heterophoxus oculatus	of authors NEP not (Holmes 1908)
Hydroides elegans	Hydroides pacificus	Hartman 1969
Keenaea centifilosum	Nemocardium centifilosum	of SCAMIT Ed 8
Kirkegaardia cryptica	Monticellina cryptica	Blake 1996
Kirkegaardia siblina	Monticellina siblina	Blake 1996
Kirkegaardia tesselata	Monticellina tesselata	of SCAMIT Ed 11
Kurtiella grippi	Rochefortia grippi	Dall 1912
Kurtiella tumida	Rochefortia tumida	of SCAMIT Ed 6
Lamispina schmidtii	Pherusa negligens	of SCAMIT Ed 11
Laonice cirrata	Laonice pugettensis	Banse & Hobson 1968
Leucothoe nagatai	Leucothoe alata	of Nagata 1965 not J. L. Barnard in Barnard & Reish 1959
Lottia instabilis	Lottia ochracea	of authors NEP
Lumbrineris ligulata	Lumbrineris californiensis	Hartman 1944
Marphysa disjuncta	Marphysa sp A	Harris & Velarde 1983 º
Megasyllis nipponica	Syllis (Typosyllis) nipponica	of SCAMIT Ed 4 list
Mesokalliapseudes crassus	Kalliapseudes crassus	Menzies 1953
Metacarcinus anthonyi	Cancer anthonyi	Rathbun 1897
Metacarcinus gracilis	Cancer gracilis	Dana 1852
Molpadia arenicola	Caudina arenicola	of SCAMIT Ed 10
Murchisonella occidentalis	Aclis occidentalis	of SCAMIT Ed 10
Neodexiospira brasiliensis	Janua (Dexiospira) brasiliensis	(Grube 1872)
Nephtys caecoides	Nephtys californiensis	of Hartman 1950 in part not Hartman 1938
Nephtys ferruginea	Nephtys californiensis	of Hartman 1950 in part not Hartman 1938
Nereis sp A	Nereis procera	of authors SCB not Ehlers 1868
Nipponnemertes rubella	Amphiporus rubellus	Coe 1905
Notopoma sp A	Cerapus tubularis Cmplx	of SCAMIT Ed 6
Nuculana sp A	Nuculana elenensis	of authors NEP not G. B. Sowerby I in Broderip & Sowerby 1833
Ophionereis eurybrachiplax	Ophionereis eurybrachyplax	of Nielsen 1932
Owenia collaris	Owenia collaris	of Hartman 1969
Oxydromus pugettensis	Ophiodromus pugettensis	(Johnson 1901)
Oxyurostylis pacifica	Oxyurostylis tertia	of authors SCB not Zimmer 1945
Paradialychone bimaculata	Chone duneri	of authors NEP not Malmgren 1867
Paradialychone ecaudata	Chone minuta	Hartman 1944
Paradialychone paramollis	Chone mollis	of authors SCB in part not (Bush 1904)
Paramicrodeutopus schmitti	Microdeutopus schmitti	Shoemaker 1942
Paranemertes sp B	Paranemertes californica	of Coe 1940 not Coe 1904
Paraonides platybranchia	Paraonella platybranchia	of Blake 1996
Paraprionospio alata	Paraprionospio pinnata	of authors NEP not (Ehlers 1901)
Parexogone acutipalpa	Exogone acutipalpa	Kudenov & Harris 1995
Parexogone breviseta	Exogone breviseta	Kudenov & Harris 1995

Table E1. (Continued).

2019 Species Name	SQO Species List Name	Synonym Describer
Parvaplustrum cadieni	Parvaplustrum sp A	SCAMIT 1995 º
Photis brevipes	Photis californica	of J. L. Barnard 1954 not Stout 1913
Piromis capulata	Pherusa capulata	(Moore 1909)
Pista brevibranchiata	Pista percyi	Hilbig 2000
Pista wui	Pista sp C	Williams 1985 º
Podarkeopsis sp A	Podarkeopsis sp A	Harris 1985 º
Poecilochaetus martini	Poecilochaetus sp A	SCAMIT 2003 º
Polyschides quadrifissatus	Siphonodentalium quadrifissatum	of SCAMIT Ed 4
Prionospio lighti	Prionospio (Minuspio) lighti	Maciolek 1985
Prionospio pygmaeus	Apoprionospio pygmaea	of authors NEP
Prosphaerosyllis bilineata	Sphaerosyllis californiensis	of Hartman 1966 in part
Psammotreta obesa	Leporimetis obesa	of SCAMIT Ed 6
Rhynchospio arenincola	Rhynchospio glutaea	of authors NEP not (Ehlers 1901)
Romaleon antennarium	Cancer antennarius	of Rathbun 1930
Romaleon jordani	Cancer jordani	Rathbun 1900
Saccella penderi	Nuculana penderi	of SCAMIT Ed 6
Scolanthus scamiti	Scolanthus sp B	MEC 1992 º
Scolanthus triangulus	Scolanthus sp A	SCAMIT 1983 º in part
Scoletoma erecta	Lumbrineris erecta	(Moore 1904)
Scoletoma tetraura Cmplx	Scoletoma luti	of authors SCB not (Berkeley & Berkeley 1945)
Semiodera inflata	Pherusa inflata	of authors NEP
Solemya pervernicosa	Solemya reidi	Bernard 1980
Spiochaetopterus costarum Cmplx	Spiochaetopterus costarum	of authors NEP not (Claparède 1870)
Spiophanes kimballi	Spiophanes berkeleyorum	of authors NEP in part not Pettibone 1962
Spiophanes norrisi	Spiophanes bombyx	of authors NEP not (Claparède 1870)
Sternaspis affinis	Sternaspis fossor	of authors NEP
Sthenelais tertiaglabra	Sthenelais verruculosa	of Pettibone 1953 not Johnson 1897
Syllis farallonensis	Syllis (Typosyllis) farallonensis	of SCAMIT Ed 4 list
Syllis gracilis Cmplx	Syllis (Syllis) gracilis	of SCAMIT Ed 4
Syllis hyperioni	Syllis (Ehlersia) hyperioni	Dorsey & Phillips 1987
Tellina cadieni	Tellina carpenteri	of authors SCB in part not Dall 1900
Tellina sp B	Tellina carpenteri	of authors SCB in part not Dall 1900
Trochochaeta franciscana	Trochochaeta multisetosa	of authors NEP not (Ørsted 1844)
Tryphosinae incertae sedis entalladurus	Uristes entalladurus	J. L. Barnard 1963
Westwoodilla tone	Westwoodilla caecula	of authors NEP not (Bate 1857)
Zeuxo normani Cmplx	Zeuxo paranormani	Sieg 1980