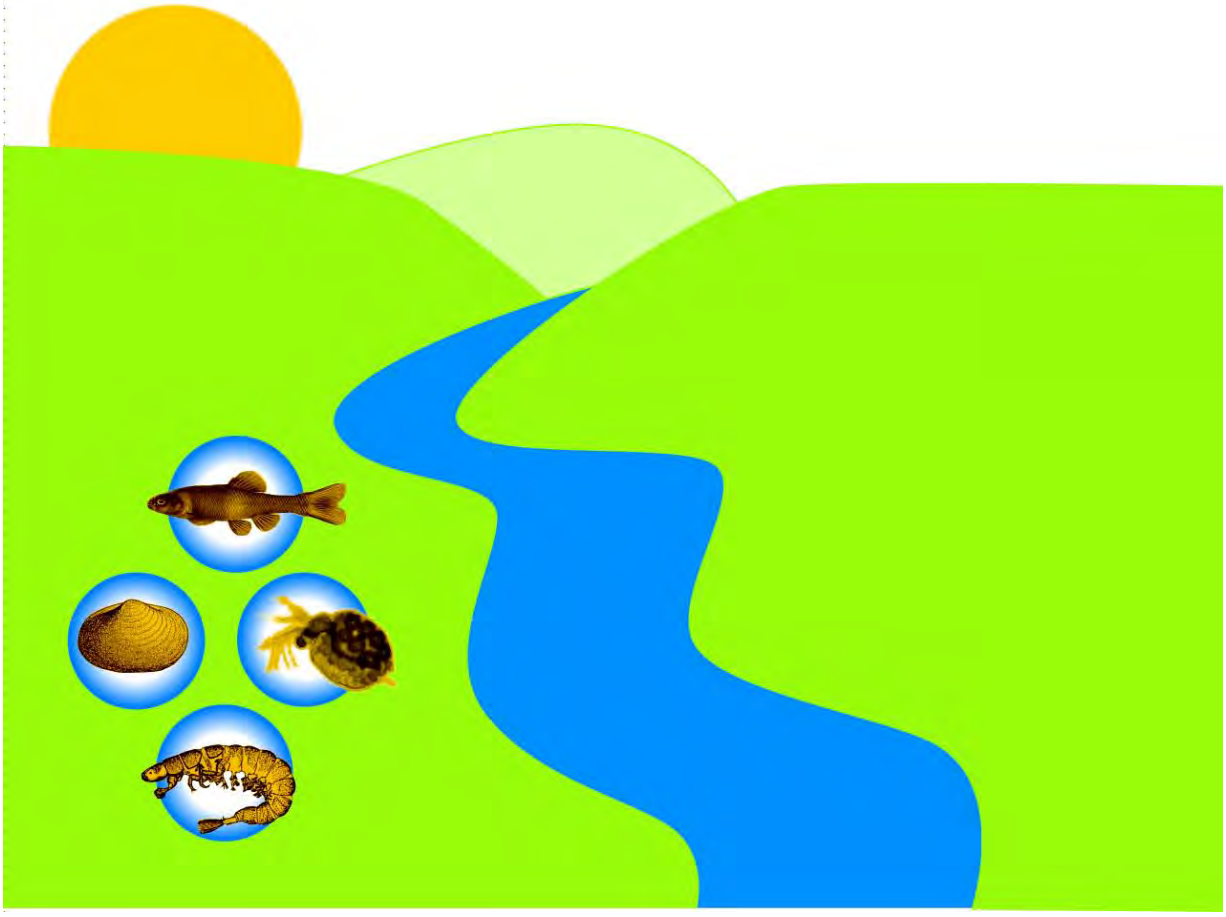
EPA United States Environmental Protection Agency

Handbook

A Compendium of Chemical, Physical and Biological Methods for Assessing and Monitoring the Remediation of Contaminated Sediment Sites



A Compendium of Chemical, Physical and Biological Methods for Assessing and Monitoring the Remediation of Contaminated Sediment Sites

EPA Contract No. 68-W-99-033
Work Assignment 4-20

Submitted to

U.S. Environmental Protection Agency

Prepared by

Battelle Memorial Institute
397 Washington Street
Duxbury MA 02332

February 17, 2003

CONTACTS

James Lazorchak and Jon Josephs are the EPA contacts for the Compendium of Chemical, Physical, and Biological Methods for Assessing and Monitoring the Remediation of Contaminated Sediment Sites. James Lazorchak, Work Assignment Manager for the preparation of the Compendium, is an Aquatic Ecotoxicologist assigned to the Ecological Exposure Research Division in Cincinnati, OH, which is under the direction of the National Exposure Research Laboratory with headquarters in Research Triangle Park, NC.

Jon Josephs, Deputy Work Assignment Manager for preparation of the Compendium, is an Environmental Engineer assigned to the Hazardous Substances Technical Liaison Program, which is under the direction of the Office of Science Policy with headquarters in Washington, DC. He is stationed at the EPA Region 2 office in New York City, NY.

ABSTRACT

Considering the many organizations which have published methods for monitoring contaminated sediments and the large number of documents on this subject, it can be a formidable task for a Superfund project manager to find methods appropriate for his or her contaminated sediment site. This Compendium of Chemical, Physical and Biological Methods for Assessing and Monitoring the Remediation of Contaminated Sediment Sites has been prepared to inform Superfund project managers and others about appropriate methods for monitoring and assessing the remediation of contaminated sediments. Although the document can be printed as a text document, it is also intended to be viewed on a computer screen in order to take advantage of its hypertext links to navigate the document and to access reference documents available on the Internet. Search engines can also be utilized to locate information contained in the document.

The methods included in this document focus primarily on published or otherwise citeable chemical, physical, and biological testing methodologies used by EPA at Superfund sites. The document summarizes the methods, including references to the methods and hypertext links to access those methods which are available on the Internet. Without exception, it is intended that all of the methods presented will be suitable for investigations at Superfund sites containing contaminated sediments. However, not all methods will be suitable for all sites. The selection of methods for a particular site will depend on the site conditions, remediation plans, budgetary constraints and other factors.

This report was submitted in fulfillment of Contract Number 68-W-99-033 by Battelle Memorial Institute under the sponsorship of the United States Environmental Protection Agency. The report was prepared during a period from July 6, 2001 to February 17, 2003 when the work was completed.

TABLE OF CONTENTS

TABLES

Table of Acronyms

ADCP	Acoustic Doppler Current Profiler
AVS	Acid Volatile Sulfide
AWQC	Ambient Water Quality Criteria
BAFS	Bioaccumulation Factors
BSAFS	Biota-Sediment Accumulation Factors
CAD	Confined Aquatic Disposal
CB	Chlorinated Biphenyl
CDD	Chlorinated Dibenzo-p-dioxins
CDF	Chlorinated Dibenzofurans
CHV	Chronic Value
COC	Constituents of Concern
CVAA	Cold Vapor Atomic Absorption
CVAFS	Cold Vapor Atomic Fluorescence Spectrometry
CTD	Conductivity, Temperature**,** Depth
CWT	Coded Wire Tags
DBT	Dibutyltin
DCM	Dichloromethane
DMA	Dimethylarsinic Acid
DO	Dissolved Oxygen
DOC	Dissolved-phase Organic Carbon
EGD	Effluent Guidelines Division
EICP	Extracted Ion Current Profile
EMAP	Environmental Monitoring and Assessment Program
EMDL	Estimated Method Detection Limit
ERL	Effects Range Low
ET	Ecotox Threshold
FAA	Flame Atomic Absorption
4BT	Tetra Butyltin
GC	Gas Chromatography
GC/ECD	Gas Chromatography *using* Electron Capture Detection

GFAA	Graphite Furnace Atomic Absorption
GPC	Gel Permeation Chromatography
HIB	Hilsenhoff's Family Biotic Index
HOC	Hydrophobic Organic Contaminants
HPLC	High-performance Liquid Chromatography
IA	Inorganic Arsenic
IBI	Index of Biotic Integrity
IC	Ion Chromatography
ICI	Invertebrate Community Index
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
INAA	Instrumental Neutron Activation Analysis
IR	Infrared
LC50	Lethal Concentration for 50%
LIR	Load Increment Ratio
LOEC	Lowest Observable Effects Concentration
MBT	Monobutyltin
MDL	Method Detection Limit
ML	Minimum Level
MMA	Monomethylarsonic Acid
MS	Mass Spectrometer
NOEC	No Observable Effects Concentration
NPDES	National Pollutant Discharge Elimination System
NTU	Nephelometeric Turbidity Units
PAH	Polycyclic Aromatic Hydrocarbons
PAR	Photosynthetically Active Radiation
PCB	Polychlorinated Biphenyl
POC	Particulate-phase Organic Carbon
PSDDA	Puget Sound Dredged Disposal Analysis
RBP	Rapid Bioassessment Protocols
SAI	Simple Autecological Indices
SCE	Sister Chromatid Exchange

SDS	Soxhlet/Dean-Stark
SEC	Sediment Exposure Chamber
SEM	Simultaneously Extracted Metal
SFG	Scope For Growth
SIM	Selected Ion Monitoring
SOP	Standard Operating Procedure
SPE	Solid-phase Extraction
SPI	Sediment Profiling Imaging
SPM	Settling Particulate Matter
STPGFAA	Stabilized Temperature Platform Graphite Furnace Atomic Absorption
TBT	Tributyltin
TEC	Threshold Effects Concentrations
TIC	Tentatively Identified Compounds
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
TCD	Thermal Conductivity Detection
VSS	Volatile Suspended Solids
XRF	X-Ray Fluorescence

1.0 INTRODUCTION

1.1 Background and Objectives

The extent and severity of chemical contaminants in freshwater and marine sediments and their impacts on ecological and human health have been studied by EPA and other federal, state, tribal, and local organizations for over the past 30 years. Over this period of time, field and laboratory tools and techniques have continuously improved and new methods have developed, and a body of institutional knowledge has been accumulated and refined regarding suitable methods for sample collection and field processing, laboratory processing and chemical analysis, and toxicology testing and bioaccumulation or other effects studies. This body of knowledge comprises methods manuals, guidance documents, standard methods, and published governmental reports, as well as published manuscripts in the scientific literature.

To disseminate information about current EPA methods and research on contaminated sediments, EPA assembled this compendium of methods. Methods presented focus primarily on published or otherwise citeable chemical, physical, and biological (toxicity and bioassessment) testing methodologies used by EPA at Superfund sites to determine the effects of chemical contaminants on aquatic life and human health. Although priority is given to those methods that have demonstrated efficacy at Superfund sites, the document also includes methods employed by other EPA and other federal and state programs at non-Superfund contaminated sediment sites.

The following agencies and programs have contributed significantly to the study of contaminated sediments and are the source of many of the methods or secondary references contained in this compendium.

Federal

- Environmental Protection Agency
  - Office of Research and Development
  - Office of Water
  - Office of Science and Technology
  - Office of Wetlands Oceans and Watersheds
  - Office of Wastewater Management
  - Office of Solid Waste and Emergency Response
  - Office of Pollution Protection and Toxics
  - Office of Pesticide Programs
  - Great Lakes National Program Office
- National Oceanic and Atmospheric Administration
  - National Status and Trends Program
  - Hazardous Material and Response Division
  - Damage Assessment and Remediation Program
- U.S. Army Corps of Engineers
  - Waterways Experiment Station
  - New England Division
  - Seattle District

- U.S. Navy
- U.S. Fish and Wildlife Service
- U.S. Geological Survey/National Biological Survey

Interagency

- National Dredging Team
- Puget Sound Dredged Disposal Analysis (PSDDA) Program

Considering the many organizations which have published methods for monitoring contaminated sediments and the large number of documents on this subject, it can be a formidable task for a Superfund project manager to find methods appropriate for his or her contaminated sediment site. To the best of our knowledge, no compilation of such methods has been prepared to date. This document summarizes many of the published methods from these agencies and programs related to the characterization of contaminated sediments and contaminated sediment sites. Additionally, related methods published as American Society for Testing and Materials Standards or Standard Methods for the Examination of Water and Wastewater (APHA 1999) are also included, as needed, to provide complete information in certain topic areas. Where a number of methods are available for a given monitoring activity, not all methods will necessarily be included in this document. If an EPA method is available, it will generally be given priority for inclusion. A method published by another Federal agency will often be included if it is significantly different from the EPA method or if an EPA method is not available. Methods published by other sources may be included if Federal methods are not available or if the methods have special merit.

The compendium is divided in four sections. The first section addresses the application and uses of monitoring data. While many of the monitoring methods have been developed for purposes other than monitoring at Superfund sites, the first section addresses applications within the Superfund decision making process, and not the broader area of marine or aquatic environmental monitoring. The monitoring methods are presented in three sections by the matrix being monitored — water, sediment, and biota. Each of these sections contains separate subsections on sampling methods and immediate field processing, chemistry and physical analysis methods, and biological analysis methods. *In situ* data collection methods are presented in the sampling methods section. Some of the methods, particularly the chemistry methods, are applicable to more than one matrix and, thus, have been presented more than once. Effort has been made in these cases to reduce redundancy as much as possible.

1.2 Application and Uses of Field, Analytical, and Testing Data at Superfund Sites Containing Contaminated Sediments

The collection of chemical, physical, and biological data at Superfund sites containing contaminated sediments is used to support human health and ecological risk assessment. To support human health risk assessment, the contaminated media to which humans may be exposed must be characterized. Exposure may be through routes such as ingestion of (the edible portions of) contaminated fish and shellfish, ingestion of contaminated drinking water, and dermal contact during swimming and wading. (Monitoring to characterize air, terrestrial species and avian species impacted by contaminated sediments is, with few exceptions, not addressed by this document.) More monitoring methods may be needed to support ecological risk assessment than to support human health risk assessment because of the variety of biota and the complexity of interactions involving contaminants and aquatic ecosystems.

Monitoring and monitoring data are important during the decision making process, which includes

the following activities:

- Site Assessment (including Preliminary Assessment and Site Inspection)
- Planning and Implementing Removal Actions
- Remedial Investigation and Risk Assessment
- Feasibility Study/Remedy Selection (following NCP criteria as the basis for decision making)
- Remedy Implementation and Monitoring Recovery

Remedial Investigation and Risk Assessment

During the initial site assessment, remedial investigation information on the extent and magnitude of chemical contamination at the site is obtained. At contaminated sediment sites, water and sediment samples are often collected and analyzed for Constituents of Concern (COCs). Because the toxicity of many contaminants is dependent upon physical properties of media , as well as the potential receptors, additional chemical, physical, and biological parameters are also collected; for example:

- *Water* – hardness, alkalinity, pH, suspended organic matter, dissolved oxygen level, dissolved organic matter, salinity, temperature, depth
- *Sediment* - pH, total organic carbon, clay content and type, grain size distribution, redox potential, depth of sample
- *Habitat structure* -- bottom characteristics, grain size distribution, cobbles, boulders, benthic community structure, fish community structure, vegetation, and debris, among others

Data on the presence of endangered or threatened species, sensitive species, and species of economic or recreational importance, and information on critical or sensitive habitats are also collected.

These data will be used in the development of a screening-level problem formulation and ecological effects evaluation and screening-level preliminary exposure estimates. Often, appropriate Ecotox Thresholds (ETs) will be used during the preliminary stages. ETs are defined as media-specific contaminant concentrations above which there is sufficient concern regarding adverse ecological effects to warrant further site investigation (USEPA 1996a). At contaminated sites, the benchmarks commonly used to assess preliminary risk (establish the ETs) include:

- *Water* – National Ambient Water Quality Criteria (AWQC). Water quality for both water and pore water is evaluated by comparison to AWQC for protection of aquatic life as specified by USEPA in numerous guidelines. These criteria, most recently updated in 1999 (USEPA 1999a), are intended to accurately reflect the latest scientific knowledge of the effects of these chemicals on aquatic life. The current AWQC list recommended criteria for 157 pollutants.
- *Sediment* – (1) Effects Range Low values (ERL) (Long and Morgan 1990, USEPA 1992a, Long et al. 1995); (2) Threshold Effects Concentrations (TECs) (MacDonald et al., 2000). ER-Ls have been developed from correlated biological and chemical

data from laboratory and field studies combined with modeling studies representing marine and estuarine environments. TECs for 28 chemicals can be used to screen freshwater sediment for risk to benthic organisms but are not necessarily protective of higher trophic level organisms.

EPA Superfund has developed an ET program that calculates site-specific ETs by adjusting for pH and hardness in surface water and TOC in sediments (USEPA 1996a). The ET calculations use as a baseline the risk-based benchmarks referenced above.

During the screening-level problem formulation/ecological effects evaluation and again during the risk characterization, the ecological significance of potential ecological receptors, as described in the *Ecological Risk Assessment Guidance for Superfund* (USEPA 1997a) is also estimated. During the problem formulation phase, ecological attributes that will function as *assessment endpoints* are identified and an assessment of the proposed endpoints is made that will help determine risk.

During problem formulation, the significance of adverse toxicological, biological, and ecological effects to receptors is considered as part of the selection process for assessment endpoints. Examples of endpoints for contaminated sediment sites include:

- *Individual Level* -Endangered or threatened species known to be present
- *Population Level* - Sensitive fish population, sensitive macroinvertebrate population, or sensitive bird population exposed to COCs
- *Community Level* - Distribution and abundance of fish and avian communities, benthic macroinfauna communities, and aquatic plant communities

Distinguishing potential and current adverse effects due to releases of contaminants at population and community levels from normal fluctuations requires knowledge of the natural variability inherent in the ecosystem (population fluctuations, presence/absence, abundance, and diversity).

During the risk characterization phase, the likelihood, duration, and magnitude of risk to the receptors represented by assessment endpoints, the spatial and temporal extent of the risk, and the estimation of COCs below which contaminants would no longer be of concern are all developed. As presented in USEPA 1994a, candidate assessment endpoints in field studies at contaminated sediment sites can include

Type	**Measurement Endpoint**
Populations	Survival and reproduction of fish Survival, growth, and reproduction of aquatic insect eating or fish-eating birds and mammals

Communities	Biomass Productivity and respiration Species richness Species density Relative abundance Dominance Diversity Evenness Similarity/difference between Superfund site and reference site Similarity/difference in guild structure between Superfund site and reference site Presence, absence, or population density of indicator species

The types of organisms/data collected and methods that may be used for collection include

Biota	**Method**
Periphyton	Scraping Coring (or sampling with a grab) Suction Artificial substrate
Plankton	Trapping Pumping Netting (towing) Water sampling
Benthic Macroinvertebrates	Dredging or digging Stream netting, sweep netting Coring (or sampling with a grab) Artificial substrate
Fish	Seining Trawling Passive netting (gill, trammel, or hoop nets) Electrofishing Chemical collection
Birds	Auditory and visual studies Nesting success Trapping

Additionally, sediment toxicity tests provide another mechanism to determine if contaminated sediments are causing adverse effects in organisms in a controlled laboratory setting. The tests that are commonly used for testing contaminated sediments include, aquatic, sediment, and microbial tests. Many are highly standardized and have the advantage of wide acceptance. Standardization has also resulted in the advantage of multiple laboratories with qualifications to

perform the testing. Aquatic toxicity tests include both freshwater and marine acute and chronic toxicity tests. Freshwater and marine sediment toxicity tests include acute and chronic tests of whole sediment or sediment interstitial water.

Several USEPA and other agency documents establishing testing methods for the suitability of dredged material for disposal in the marine and freshwater environments have been prepared (USEPA/USACE 1991, USEPA/USACE 1992, USEPA/USACE 1998, USEPA 1994a, USEPA 2000a, USACE/WDNR/WDEC 2000). While these documents do not specifically address the Superfund program, they present the various approaches taken for the management of contaminated sediments, including the use of both sediment chemistry and toxicity data in contaminated sediment evaluation.

Remedy Selection/Feasibility Studies

While monitoring and field data acquisition can occur in the above phases of the Superfund risk assessment process, the field data will also need to be acquired for the remedy selection. The remedies to be considered at contaminated sediment sites include

- Monitored natural recovery
- Containment in-place (*in situ* capping)
- Treatment in-place
- Removal and disposal of contaminated sediments in confined aquatic disposal (CAD) facility
- Removal and upland containment.

The extent to which one of these remedies is superior needs to be determined on a case by case basis based on site data, appropriately acquired following sound project planning and the application of sound monitoring methods and data assessment techniques. As presented in Sediment Management Work Group (1999), the appropriate questions that need to be answered to guide remedial at contaminated sediment sites include

- How long will it take natural recovery to return the site to acceptable conditions?
- Will dredging and removal accelerate this process?
- Will other remedial options (*i.e.*, capping) accelerate the process?
- What are the risks (for example, from large storm events) of leaving the contaminated sediment in place, or of removing the engineered clean sediment cap?

The answers to the above questions can be derived from an understanding of the site from site characterization studies, the appropriate application of transport and fate models and ecological and human health risk assessments, leading to the development of a site conceptual model of the transport and fate of COCs at the site that will lead to a risk-based remedy decision.

Specific site condition information needs to be collected and evaluated when considering an *in situ* capping remediation alternative at contaminated sediment sites, including:

- *Physical environment* - For example, bathymetry will influence the ability to place an *in situ* cap in many areas and will also influence dispersion at the site during dredging or capping. Bottom slope needs to be considered. Moderate slopes can rule out the ability to cap without the placement of a physical barrier to prevent downslope movement.

- *Hydrodynamic conditions* - Stability of the cap over time (typically 30 years) needs to be determined. This determination will require data such as normal water column currents or channel flow, tidal fluctuations, wave and storm induced bottom currents, or flow during flood conditions need to be considered. Some of these data will feed into modeling studies, which may be needed to understand conditions at open water sites.

- *Hydrogeological conditions* - Groundwater discharge to near shore areas of lakes, rivers and estuaries is common in many areas of United States and groundwater flow through a contaminated sediment site can transfer a fraction of the contaminants to the overlying surface water. In this circumstance, a determination of the magnitude of groundwater flow and thickness of the contaminated layer should be made. Additionally, data on sediment physical characteristics, such as water content, grain size and clay content and type, organic content, plasticity indices, and specific gravity, are also required to evaluate site conditions and cap design. Engineering measurements of sediment shear strength and compressibility are also required.

Monitoring activities associated with the evaluation of fate and transport processes for site assessment, risk characterization, remedy selection, or post remedy monitoring can include those with the purpose of:

- Characterizing stream flow and the potential for sediment deposition and/or scouring

- Assessing naturally occurring biodegradation of contaminants (which may include methods to characterize sediment geochemistry, biodegradation products, microbial populations, chiral analytes, *etc.*)

- Assessing diffusive transport of contaminants through sediments to the water column

- Assessing water flow across the sediment/water column interface

- Assessing the consolidation of sediments resulting from compressive forces and biodegradation

- Determining changes in the location of the sediment/water interface

- Assessing the mobility of contaminants (*e.g.*, speciation methods for metals, total organic carbon, acid volatile sulfides, pH, redox potential, *etc.*)

- Characterizing the physical characteristics of sediments (*e.g.*, sediment cohesion, shear strength, particle size distribution, *etc.*)

Monitoring activities facilitating the implementation of remedial actions can include those with the purpose of:

- Evaluating monitored natural recovery (*e.g.*, by decrease in concentrations of COCs, decrease in toxicity
- Facilitating installation of *in situ* caps
- Determining the long-term performance and condition of *in situ* caps (*e.g.*, by measuring cap thickness over time, measuring COCs in sediment cap porewater over time)
- Evaluating aquatic system recovery following installation of *in situ* caps
- Facilitating remedial dredging
- Evaluating aquatic system recovery following completion of remedial dredging
- Ensuring that remedial construction activities do not have the potential to produce and are not producing immediate adverse effects (*e.g.*, by monitoring turbidity, dissolved oxygen, acute toxicity testing)

The methods presented in the next several sections are frequently used for assessment of environmental conditions at contaminated sediment sites and provide data to address many of the data uses described above. Many of these methods were originally developed for the analysis of water and wastewater or for the analysis of solid waste have been used without modification for marine or aquatic investigations. Other methods originally developed for those purposes have been modified for contaminated sediment investigations, often to lower detection limits needed for risk assessments or to facilitate working with a high salinity water or sediment matrix. However, many of the methods were specifically developed for working in the aquatic or marine environment at contaminated sites, and elsewhere. Without exception, it is intended that all of the methods presented will be suitable for investigations at Superfund sites containing contaminated sediments. However, not all methods will be suitable for all sites. The selection of methods for a particular site will depend on the site conditions, remediation plans, budgetary constraints and other factors.

2.0 MONITORING METHODS

2.1 Water

Water sample collection methods, and chemical, physical, and biological analyses have continuously been developed and implemented over the years to evaluate the health of our nations streams, lakes, rivers, ponds, creeks, lagoons, estuaries, oceans and surface impoundments.

At Superfund sites, water samples are specifically collected with the following objectives in mind (USEPA, 1994a):

- To determine if the contaminant is hazardous by identifying its composition and characteristics;
- To determine if there is an imminent or substantial threat to public health or welfare or to the environment;
- To determine the need for long-term action;
- To develop containment and control strategies;
- To evaluate appropriate disposal/treatment options; and,
- To verify treatment goals or clean up levels.

In order to achieve the aforementioned objectives, field sampling and analytical strategies are designed to provide site-specific information because characteristics and sampling strategies vary widely. The following fact sheets relating to water are divided into sections pertaining to field sample collection and processing, chemical and physical laboratory analyses and biological laboratory analyses. These fact sheets intend to provide Superfund managers with a summary of the existing methods that may be applicable to their site, their relative strengths, and their relative weaknesses.

2.1.1 Field Sample Collection and Immediate Processing, *In Situ* Data Acquisition

Section 2.1.1 presents methods for field sample collection, field or immediate sample processing, and *in situ* data acquisition. *In situ* data acquisition primarily collects those data easily and economically collected with various sensors and includes parameters such as salinity, conductivity, temperature, pH, dissolved oxygen, light transmission, and light attenuation. Data collection *in situ* is often automated and allows for data acquisition over broad spatial and temporal scales. Because many biological processes in the environment are affected, directly or indirectly, by the physical characteristics of the environment, the data collected *in situ* are vital in site assessments at aquatic sites. Water samples are also collected in numerous ways and brought back to the laboratory to identify chemical contaminants that may disrupt existing physical and biological processes and what effects those chemical contaminants may have on resident organisms. These samples and analyses are crucial in determining potential exposure pathways based on the concentration of the chemical in the environment and then the chemical's behavior in the environment based on biological, chemical, and physical parameters. Measurement and collection methods vary based on the characteristics of the water body in question. Therefore, numerous methods are provided.

Many of the field sampling and data collection methods for water are routinely performed to evaluate the health and biological integrity of our surface waters. Thus, they originate from programs unrelated to contaminated sediments. Many of the methods have been taken from USEPA Environmental Monitoring and Assessment Program documents. Other sources of methods for water column field collection include:

- The USEPA and the Puget Sound Water Quality Action Team
- The USEPA's Lake Michigan Mass Balance Study Methods Compendium
- The USEPA's Environmental Response Team
- The USEPA's Environmental Research Laboratory-Narragansett
- Standard Methods for Examination of Water and Wastewater
- ASTM
- Journal publications
- The USEPA's Rapid Bioassessment Protocols

Fact Sheet No.	2.1.1-1	
Method Title	*In Situ* Sampling with the Hydrolab Datasonde3® Unit	
Purpose	This procedure describes one of several CTD devices used to collect high-quality *in situ* data for salinity, temperature, dissolved oxygen (DO) concentration, pH, and water depth.	
Method Summary	The Hydrolab Datasonde3® unit is one of several commercial units that collect *in situ* Conductivity, Temperature, and Depth (CTD) data, and also measures pH and DO to obtain a vertical profile of water column conditions. After calibration, the Hydrolab is connected to a winch cable, and the protective covers on the probes are removed and the stirrer is connected. The unit is lowered over the side and allowed to equilibrate at the surface for at least two minutes. While the unit is equilibrating, a QC check is performed with a YSI DO probe, refractometer and a thermometer to ensure that the readings from all equipment agree. The Hydrolab unit is lowered at intervals specific to the relative depth of the site, allowing the unit to stabilize at each specified stop during descent. Data can be acquired on the descent and ascent.	
Data Uses/Application	Salinity, conductivity, temperature and dissolved oxygen parameters are commonly collected at all stations where samples for water quality are collected.	
Advantages	The Hydrolab is a relatively small unit that may be hand deployed, if necessary.	
Limitations	The Hydrolab is much more expensive than alternate sampling equipment such as the YSI data probe, refractometer and thermometer. Without proper calibration, the Hydrolab or other CTD units may record erroneous information.	
Reference	Strobel, C.J. 2000. Coastal 2000 - Northeast Component: Field Operations Manual. U.S. Environmental Protection Agency , National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett, R.I. EPA/620/R-00/002.	
Website	http://www.epa.gov/emap/nca/html/docs/c2knefm.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-2	
Method Title	*In Situ* Dissolved Oxygen Sampling with a YSI Model 58 Dissolved Oxygen Meter and Probe	
Purpose	This method describes the use of a YSI Dissolved Oxygen Meter and Probe to take *in situ* oxygen measurements in the water column.	
Method Summary	After checking the batteries and replacing the probe membrane, the YSI Model 58 Dissolved Oxygen(DO) Meter and Probe must be calibrated before sampling at each station. The meter is then set to 0.1 mg/L mode. If measuring the DO in other than surface water, collect water in a Go-Flo® bottle. Measure the salinity with a refractometer. Insert the stirrer-probe unit directly into the Go-Flo bottle and turn on the stirrer. When the meter reading has stabilized, record the oxygen value on the data sheet. Turn the stirrer off, rinse the probe with freshwater, and store the unit out of sunlight.	
Data Uses/Application	DO is an important water quality parameter for surface water aquatic life. The YSI Model 58 Dissolved Oxygen Meter and Probe can be used to take oxygen measurements at the surface as a Quality Control check on DO measurements determined by other methods. Probes are used mainly on *in situ* instruments for providing continuous water-column profiles of dissolved oxygen.	
Advantages	The YSI Model 58 Dissolved Oxygen Meter and Probe is small and transportable. It is a good, quick way to check on the accuracy of the Hydrolab. The measurement of DO directly in sampling bottles reduces sampling bias associated with sample transfer.	
Limitations	The device must be calibrated at every station prior to use. The device could produce erroneous data if the membrane is at all damaged or dried out. The probe method is not commonly used for oceanographic studies in which measurements are made on discrete samples of seawater.	
Reference	Strobel, C.J. 2000. Coastal 2000 - Northeast Component: Field Operations Manual. U.S. Environmental Protection Agency , National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett, R.I. EPA/620/R-00/002.	
Website	http://www.epa.gov/emap/nca/html/docs/c2knefm.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-3	
Method Title	*In Situ* Irradiance Measurements	
Purpose	This method describes the manner in which a vertical profile of light intensity can be measured for the purpose of calculating a light attenuation coefficient at each station.	
Method Summary	Photon flux, or Irradiance is the amount of light that diffuses the water referenced to the input. Photosynthetically active radiation (PAR) is measured to determine the absorption rate of that light by phytoplankton. To obtain a PAR profile, a deck sensor and an underwater sensor are connected to an independent data logger. The deck sensor is placed in a location where it is not shaded. The underwater sensor is lowered on the sunny side of the boat to a depth of about 10 cm (representing surface). Record the values from both sensors. Lower the underwater sensor to 0.5 meters and record the values. Then lower the sensor at given intervals depending on the relative depth of the sampling location and record the values. Repeat the process on the upcast.	
Data Uses/Application	PAR is important in understanding the dynamics of the "photic zone" which helps to understand lake health issues such as photosynthesis, and toxic algae blooms and eutrophication. Irradiance and PAR are measured for the purpose of calculating a light attenuation coefficient at each station which can be used in productivity assessments or for site assessments.	
Advantages	PAR sensors require no field calibration, but they should be returned to the manufacturer prior to each field season for annual calibration. PAR sensors provide more quantitative data to derive light attenuation data in a more exact manner.	
Limitations	PAR sensors are more expensive than other light attenuation methods.	
Reference	Strobel, C.J. 2000. Coastal 2000 - Northeast Component: Field Operations Manual. U.S. Environmental Protection Agency , National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett, R.I. EPA/620/R-00/002.	
Website	http://www.epa.gov/emap/nca/html/docs/c2knefm.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-4	
Method Title	*In Situ* Transparency Measurements, Secchi disk profile	
Purpose	This method describes the manners in which a vertical profile of light can be measured for the purposes of determining water column transparency.	
Method Summary	To obtain a Secchi profile, a 20 cm black and white Secchi disk is lowered until it is no longer visible. Note the depth using the markings on the line. Slowly raise the disk until it just becomes visible and note the depth. Repeat this process 3 times and record the average of the readings.	
Data Uses/Application	The Secchi disk measures water column transparency and may be best used when transparency is high.	
Advantages	The Secchi disk can be used on any vessel, and computer data loggers are not necessary.	
Limitations	Secchi disks data may vary amongst investigators. More room for human error.	
Reference	PSWQAT. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound, Puget Sound Protocols and Guidelines. Puget Sound Water Quality Action Team, Olympia, WA.	
Website	http://wwwpsat.wa.gov/Publications/protocols/protocol.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-5
Method Title	Sample Collection Procedures for Marine Water
Purpose	This method describes the methods and different type of water bottles used to collect marine water column samples.
Method Summary	Water samples should be collected prior to "bottom related" activities (sediment grabs and trawling) to assure that bottom sediments are not re-suspended. These samples should also be collected on a day when it is possible to ship samples on the same or following day to meet toxicity testing holding times, if necessary. The typical water bottle sampler consists of a cylindrical tube with stoppers at each end, and a closing device that is activated from the surface by a messenger or an electrical signal. Multiple water samplers can be attached sequentially to a vertical hydrowire for sampling at multiple depths on a single cast, or they can be mounted on a rosette frame (see fact sheet 2.1.1.-6; often in conjunction with an *in situ* sensor array) which allows for collection of replicate samples at the same depth. After the sampler is cocked, it is lowered to a designated depth. Avoid deploying water bottles in surface slicks as these can contaminate samples with organic compounds. If contamination by the microlayer is of concern, use samplers that are designed to remain closed until they have descended below the microlayer (*i.e.,* Go-Flo™ bottle from General Oceanics, Inc. Miami, Florida). Teflon™-lined Go-Flo™ bottles are recommended when sampling marine water that will be analyzed for ambient or trace levels of mercury or other metals upon proper cleaning Niskin bottles are also often used to collect water samples, however these bottles remain open as they descend through the water column, and the enclosure mechanism is on the inside of the bottle, making them difficult to clean. Once the sampler reaches the desired depth, it should be allowed to equilibrate to ambient conditions for approximately 1 minute before it is closed. If reversing thermometers are involved, equilibration should be 5 minutes. After equilibrium, the closing device is activated by a messenger or electric signal, and the sampler is retrieved. To ensure that all samples are truly representative of the water column within a specific water parcel, it is advisable that they be collected from a single cast. As the water samplers are being brought on board, each bottle should be checked immediately for leakage of sample water around the seals. If the sample has been compromised, the cast should be repeated.
Data Uses/Application	Water bottle samplers are used to collect water column samples for laboratory analyses of conventionals, metals, organics and microbiological analytical procedures and toxicity tests

Fact Sheet No.	2.1.1-5 (contd.)	
Advantages	Both Niskin and Go-Flo bottles can be deployed individually or attached to a large rosette sampler which collects *in situ* data while water samples are being collected. The Go-Flo design allows it to be deployed (and returned) in the closed position, reducing the possibility of sample contamination from surface slicks and the microlayer. These bottles also have external springs therefore there is no risk of sample contamination. The Go-Flo bottles are commonly used to collect samples later analyzed for metals or organics. The EMAP Program provides similar guidance regarding marine water collection (USEPA, 1990a).	
Limitations	Holding times for collecting samples must be observed. Niskin bottles are generally not used to collect samples later analyzed for sensitive organics or metal analyses since they are deployed in the open position and the spring used to close the cap is located on the inside of the bottle.	
Reference	PSWQAT. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound, Puget Sound Protocols and Guidelines. Puget Sound Water Quality Action Team, Olympia, WA.	
Website	http://wwwpsat..wa.gov/Publications/protocols/protocol.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-6	
Method Title	Field Sampling Using Rosette Sampler, LMMB 013	
Purpose	This procedure describes the collection of water samples using a Rosette Sampler.	
Method Summary	The Rosette sampler is the primary sampling instrument for the collection of all nutrient parameters, phytoplankton, chlorophyll a, Phaeophytin a, and dissolved oxygen, temperature, total suspended solids, turbidity, specific conductance, and pH. The system consists of 12 water sampling bottles (8 L Niskin or Go-Flo bottles), a CTD (conductivity, temperature and depth sensor) attached at the bottom of the Rosette, an A-frame, a multiconductor cable, a variable speed winch, and a deck unit with attached computer. The bottles can be closed in any predetermined order, remotely from the deck of the vessel while the array is submerged at the various sampling depths. The CTD is built to provide real time information on a number of water quality parameters as it moves through the water column. During sampling, the Rosette/CTD system is lowered to the bottom to define the temperature profile of the water column. The sampling depths are then selected. After collection of samples with the Rosette sampler, the sampler is brought on board and the water samples are transferred from the Niskin or Go-Flo bottles to various sample bottles, depending on analysis, for storage until processing and analysis. During sampling, each bottle is rinsed with sample water, emptied, and filled with sample water. The cap is replaced after addition of the preservative, or immediately if no preservative is added. Dissolved oxygen samples are processed immediately.	
Data Uses/Application	Rosette samplers are typically used in open water, both freshwater and marine environments, where samples from multiple stations and depths will be required.	
Advantages	Real time *in situ* water column data can be collected to determine the best depths for water sample collection. Each discrete water sample has a corresponding set of water quality parameter data.	
Limitations	Dissolved nutrient samples must be filtered before analysis, and must be filtered within one hour of collection.	
Reference	USEPA. 1997b. Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/mbross.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-7	
Method Title	Water Sample Collection with the Kemmerer Bottle and the Bacon Bomb Sampler, ERT SOP #2013	
Purpose	Both the Kemmerer Bottle and the Bacon Bomb Sampler can be used to collect surface water in situations where site access from a boat or structure is available and where samples at depth are required.	
Method Summary	A properly decontaminated, preset Kemmerer bottle or Bacon Bomb Sampler may be lowered to a predetermined depth to collect an aqueous sample. The Kemmerer Bottle can be used for general purpose sampling at the surface or at specified depths. The all-angle head locks the sampler in the open position and unlocks when struck by the messenger, closing both end seals of the sampler. The sampler is retrieved and samples are transferred to appropriate sampling container. The Bacon Bomb Sampler opens when a protruded plunger strikes bottom. It closes as the sampling device is hoisted back into the water column. By attaching a cord to the upper end of the plunger, samples may also be taken from an intermediate level as well. The sampler is then retrieved and the sample is transferred to the appropriate sampling container.	
Data Uses/Application	These samplers can collect any sort of water sample that then may be used for chemistry analysis, nutrients analysis, or toxicity studies.	
Advantages	These samplers can collect samples from a range of depths. The Kemmerer bottle has few moving parts and needs little maintenance.	
Limitations	The sampling stations need to be accessible by boat or from land. The samples could be cross contaminated if sample equipment is not appropriately decontaminated in between stations.	
Reference	USEPA. 1994b. Surface Water Sampling, SOP # 2013, in Compendium of ERT Standard Operating Procedures. Environmental Response Team. Compendium of ERT Standard Operating Protocols. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison NJ.	
Website	http://www.ert.org/products/2013.pdf	Accessed: 1/31/2003

Fact Sheet No.	2.1.1-8
Method Title	Dip Sampler, ERT SOP # 2013
Purpose	The Dip sampler is generally used to collect surface water samples in a situation where the sample is to be recovered from an outfall pipe or along a lagoon bank where direct access is limited.
Method Summary	The dip sampler is extended into the sample location to collect sample. The sampler is retrieved and the sample is transferred to the appropriate container.
Data Uses/Application	These surface water samplers can collect any sort of liquid sample that then may be used for water chemistry analysis, nutrients analysis, or toxicity studies.
Advantages	The long handle on dip samplers allows them to collect samples from discrete locations. Dip samplers are extremely durable. They can be useful in weedy habitats where other samplers may not work.
Limitations	The sampling location must be within reach of the investigator. The samples could potentially be cross-contaminated if the sampler is not properly cleaned between sample collections.
Reference	USEPA. 1994b. Surface Water Sampling, SOP # 2013, in Compendium of Environmental Response Team Compendium of Standard Operating Procedures. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison NJ.
Website	http://www.ert.org/products/2013.pdf Accessed: 1/31/2003

Fact Sheet No.	2.1.1-9
Method Title	Sample and Preservation of Water Specific Parameters
Purpose	This method describes the sample collection procedures unique to conventionals, metals, organics, and microbiological analytical parameters. See Table 2.1.1-1 for details pertaining to recommended sample sizes, containers, preservation techniques and holding times for water.
Method Summary	For analysis of conventional parameters, those parameters subject to biological alteration should be measured first and within 15 minutes of sampling. Dissolved oxygen should be the first parameter collected, followed in order of priority by those parameters which would be the most affected by subsampling delays. For analysis of total metals or total mercury, the samples should be acidified to pH <2 using ultrapure HNO_3. Samples that will be analyzed for mercury speciation should be preserved with HCl rather than HNO_3. Samples that will be analyzed for both dissolved and particulate metals should be filtered as soon as possible, within 24 hours of collection. The filtrate, which contains the dissolved fraction, should be preserved by acidifying to pH<2 using ultrapure HNO_3. The particulate fraction, which is retained on the filter, is frozen for preservation. For organics analysis, the samples for analysis of volatile organic compounds are collected first in 40 ml VOA vials leaving no head space. The samples should be protected from possible contamination such as fuels, winch grease, exhaust, and solvents that may be present on or around a research vessel. Preserve water samples collected for organics analysis as soon as possible, according to the guidelines summarized in the attached table. For microbiological analyses, it is important to collect from the microlayer, or surface-most layer of water. The microlayer is most easily included when using the scoop method. The scoop method involves plunging an open bottle straight down to a depth of 15 to 39 cm below the water surface, moving it horizontal to the surface while tipping it slightly to let trapped air escape, and removing the bottle in a vertical position. Approximately 2.5 cm of head space is required in the sample container. Sample containers should be isolated from contact with wet ice as it could impart contamination to the sample.
Data Uses/Application	These samples are then shipped to analytical laboratories where the aforementioned analyses are performed to determine water chemistry.
Advantages	Filtration is used for metals analysis since it is inexpensive and yields a sample that is directly suitable for chemical analysis.
Limitations	Holding times must be met in order to appropriately analyze the samples. Care must be taken to avoid any sort of contamination of these samples since they will be analyzed with sensitive procedures. Small amounts of particulate metals are collected during filtration, which make it difficult to use them for low-level metal analyses.

Fact Sheet No.	2.1.1-9 (contd.)	
Reference	PSWQAT. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound, Puget Sound Protocols and Guidelines. Puget Sound Water Quality Action Team, Olympia, WA.	
Website	http://www.psat.wa.gov/Publications/protocols/protocol.html	Last Accessed: 1/31/2003

Table 2.1.1-1. A Summary of Sample Sizes, Containers, Preservation Techniques, and Holding Times for Water (PSWQAT. 1997.)[1]

Parameter	Minimum Sample Size (ml)	Container	Preservation Technique	Holding Time
Alkalinity	100	Glass or Polyethylene	Refrigerate, 4° C	14 Days
Total Hardness	100	Glass or Polyethylene	Refrigerate, 4° C HNO_3 to pH<2	6 Months
Total Phosphorous	50	Glass or Polyethylene	Refrigerate, 4° C $H_2SO_4$to pH<2	28 Days
Orthophosphate	50	Glass or Polyethylene	Refrigerate, 4° C Filter on site	48 Hours
pH	25	Glass or Polyethylene	None	Analyze Immediately
Salinity	200	Glass or Polyethylene	None	28 Days
Turbidity	100	Glass or Polyethylene	Refrigerate, 4° C	48 Hours
Total Suspended Solids	1,000 to 4,000	Glass or Polyethylene	Refrigerate, 4° C	7 Days
Dissolved Oxygen Winkler	125	Glass Bottle with Glass Top	Fix with $MnCl_2$ and Alk. Iod. (2 ml ea.)	8 Hours (store in the dark)
Dissolved Oxygen Probe	125	Glass Bottle with Glass Top	None	Analyze Immediately
Ammonia-N	100	Glass or Polyethylene	Refrigerate, 4° C $H_2SO_4$to pH<2	28 Days
Nitrite-N	100	Glass or Polyethylene	Refrigerate, 4° C	48 Hours
Nitrate-N	100	Glass or Polyethylene	Refrigerate, 4° C	48 Hours
Silica	200	Polyethylene	Refrigerate, 4° C	28 Days
Chlorophyll a	25 to 1,000	Glass or Polyethylene (Dark)	Store filters frozen (-20 ° C) in the dark	28 Days
Volatile Organics	80	Glass -2 40 ml vials, No Head space	Refrigerate, 4° C HCl to pH<2	14 Days

Table 2.1.1-1. (contd.)

Parameter	Minimum Sample Size (ml)	Container	Preservation Technique	Holding Time
Semi-volatile Organics	1,000 to 2,000	Glass	Refrigerate, 4° C	7 Days
Total Mercury and Diss. Mercury	500	Teflon™ or Glass with Teflon™ Cap	Refrigerate, 4° C HNO_3 to pH<2	28 Days
Total Metals and Diss. Metals	1,000	Polyethylene or Teflon™	Refrigerate, 4° C HNO_3 to pH<2	6 Months
Microbiology	500	HDPE (Autoclaved)	Refrigerate, 4° C	24 Hours

[1] PSWQAT. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound, Puget Sound Protocols and Guidelines. Puget Sound Water Quality Action Team, Olympia, WA.

Fact Sheet No.	2.1.1-10	
Method Title	Sampling of Particulate-Phase and Dissolved-Phase Organic Carbon in Great Lakes Waters, LMMB 014	
Purpose	This method describes the sampling of water for particulate-phase organic carbon (POC) and dissolved-phase organic carbon (DOC).	
Method Summary	Water samples are collected using either a submersible pump or Rosette sampler (see Fact sheet 2.1.1-6). The volume of sample to be filtered is measured in a graduated cylinder. Prior to filling, the cylinder is rinsed twice with sample water. The water sample for POC/DOC analysis is vacuum filtered through an ashed 47 mm diameter glass fiber filter (0.7 µm pore-size) in an all-glass filtration apparatus. Samples are filtered simultaneously in duplicate. The samples are acidified with 0.2 N HCl during the filtration to remove inorganic carbonates. The POC is retained on the filter, and the DOC is collected in the filtrate. The volume of sample required to produce a reliable POC measurement varies with station location, depth, and time of year. If the filter becomes visibly loaded with particles and the flow of water through the filter slows considerably, sufficient particulate matter has been collected. The POC filter is folded, placed in an aluminum foil pouch, and frozen at -10°C until analysis. The filtrate is collected and promptly analyzed for DOC in a shipboard laboratory.	
Data Uses/Application	POC/DOC are parameters which are ancillary to the determination of hydrophobic organic contaminants (HOCs).	
Advantages	POC and DOC are important water quality measurements that add to the value of water column assessments.	
Limitations	Shipboard or field processing is generally required to meet holding times for these analyses.	
Reference	USEPA. 1997b. Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/pocdoc2.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-11	
Method Title	Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels, EPA Method 1669	
Purpose	This method is for the collection and filtration of ambient water samples for subsequent determination of total and dissolved metals (antimony, arsenic, cadmium, chromium (III and VI), copper, lead, mercury, nickel, selenium, silver, thallium, zinc).	
Method Summary	Method 1669 is "performance-based"; *i.e.*, an alternate sampling procedure or technique may be used, so long as neither samples nor blanks are contaminated when following the alternate procedures. Before samples are collected, all sampling equipment and sample containers are cleaned in a laboratory or cleaning facility using detergent, mineral acids, and reagent water. After cleaning, sample containers are filled with weak acid solution, individually double-bagged, and shipped to the sampling site. If samples are to be collected for determination of trivalent chromium, the sampling team processes additional QC aliquots are processed. Upon arrival at the sampling site, one member of the two-person sampling team is designated as "dirty hands"; the second member is designated as "clean hands." All operations involving contact with the sample bottle and transfer of the sample from the sample collection device to the sample bottle are handled by the individual designated as "clean hands." "Dirty hands" is responsible for preparation of the sampler (except the sample container itself), operation of any machinery, and for all other activities that do not involve direct contact with the sample. All sampling equipment and sample containers used for metals determinations must be nonmetallic and free from any material that may contain metals. Sampling personnel are required to wear clean, nontalc gloves at all times when handling sampling equipment and sample containers. Samples for dissolved metals are filtered through a 0.45 µm capsule filter at the field site. After filtering, the samples are double-bagged and iced immediately. Acid preservation of samples is performed in the field or in the laboratory. Field preservation is necessary for determinations of trivalent chromium.	
Data Uses/Application	This method is applicable for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.	
Advantages	Clean sampling methods reduce/eliminate bias associated with sample collection handling.	
Limitations	Samples may become contaminated by numerous routes. These methods are only applicable for trace metal contaminants.	
Reference	USEPA. 1997b. Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques. EPA-821-C-01-001. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-12	
Method Title	ESS Method 340.2: Total Suspended Solids, Mass Balance (Dried at 103-105°C) Volatile Suspended Solids (Ignited at 550°C), in LMMB 065	
Purpose	To measure the portion of total solid retained by a filter from drinking, surface, and saline waters; domestic and industrial wastes.	
Method Summary	Water samples are collected by submersible pump or Rosette sampler (see Fact sheet 2.1.1-6). A sample volume is selected that will yield 2 - 20,000 mg/L. total suspended solids. For open-lake oligotrophic conditions, 2-4 liters will provide enough particulate matter. For near-shore or eutrophic conditions, 200-500 ML may be sufficient. A well-mixed sample is filtered through a preweighed standard glass-fiber filter, and the residue retained on the filter is dried at 103 to 105 °C for at least one hour. The increase in weight of the filter represents the total suspended solids. If measuring TSS of estuarine water, the filter must be well rinsed with DI water to remove salt residue. Following Method LMMB 098, water samples can be filtered in the field and then frozen at -10°C until final weighing in the laboratory. The Environmental Research Laboratory - Narragansett (NHEERL-AED) SOP 1.02.004 and Standard Method 2540D also describe similar methods for measuring total suspended solids (USEPA and Naval Construction Battalion Center, 1992; APHA, 1999).	
Data Uses/Application	TSS is an ancillary parameter to the determination of hydrophobic organic contaminants (HOCs). TSS is also commonly measured to assess water clarity or to assess sediment transport and to normalize total (aqueous) contaminant data.	
Advantages	The samples may be filtered in the field or in portable laboratory facilities.	
Limitations	Excessive residue on the filter may form a water-entrapping crust. Sample size should be limited to yield no more than 200 mg residue.	
Reference	USEPA.1997c. Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chemistry, and Biomonitoring Sample Analysis Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/methd340.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-13	
Method Title	*In situ* Peepers	
Purpose	This method describes an *in situ* method to collect pore water.	
Method Summary	A peeper, as described in this method, is a mesh-sided chamber that is inserted into the sediment for the purpose of collecting pore water. Peepers are placed *in situ* below the sediment surface with only the tubes emerging above the surface. The chambers (constructed using 500-ml polyethylene bottles, mesh sides, tygon tubing and a 50-ml syringe that slowly extracts the pore water) are buried in shallow waters and sediment packed around the unit to ensure overlying waters are not in contact with side mesh windows. Immediately following burial, water in the chamber is evacuated to enhance entry and equilibration of pore water. After several minutes, day-0 samples are collected. The syringe is capped and returned to the laboratory for analysis of the extracted water. Pore water is collected on day-0 and over predetermined time periods of up to a month.	
Data Uses/Application	*In situ* peepers are used to collect pore water for toxicological testing. The device may also be modified to suitable collect samples for chemical contaminant analyses.	
Advantages	*In situ* sampling reduces sampling and laboratory-related errors that may affect organism response (*i.e.,* resuspension of sediments that organisms would otherwise not be exposed to).	
Limitations	There may be site-specific limitations that would prevent the deployment of a peepers.	
Reference	Sarda, N. and G.A. Burton. 1995. Ammonia Variation in Sediments: Spatial, Temporal, and Method-Related Effects. *Environmental Toxicology and Chemistry*. Vol. 14: 9.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.1.1-14	
Method Title	Suction Samplers	
Purpose	Suction samplers can be used to collect water that is then analyzed for various chemicals of concern. This method is suitable for pore water samples analyzed for ammonia.	
Method Summary	The suction method uses an aquarium stone and a hand-operated vacuum pump to extract pore water from surficial sediment *in situ*. The air stone is buried under the surface of the sediment and the suction is applied. An imposed vacuum sucks pore water into an in-ground porous cup. In simple systems, the water is stored in the suction cell and is subsequently sucked or blown into a sample flask placed on the ground surface. The vacuum is not maintained between samples.	
Data Uses/Application	Suction samplers are able to extract pore water *in situ* for chemical analysis.	
Advantages	*In situ* sampling reduces sampling and laboratory-related errors that may affect organism response (*i.e.,* resuspension of sediments that organisms would otherwise not be exposed to).	
Limitations	There may be site-specific limitations that would prevent the deployment of a suction sampler.	
Reference	Sarda, N. and G.A. Burton. 1995. Ammonia Variation in Sediments: Spatial, Temporal, and Method-Related Effects. *Environmental Toxicology and Chemistry*. Vol. 14: 9.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.1.1-15	
Method Title	Physical Characterization of a Stream	
Purpose	These methods describe the data needed to characterize the physical attributes of a stream needed for a habitat assessment.	
Method Summary	Physical characterization of stream habitat includes descriptions of the: • General stream characteristics such as an analysis of the stream subsystem (where relevant), stream type (*i.e.,* cold-water vs. warm-water), and stream origin (*i.e.,* glacial, montane, swamp, bog); • Watershed features such as the predominant land use type surrounding the stream, local watershed nonpoint source pollution, and local watershed erosion; • Riparian vegetation up to 18 meters from the stream bed; • Instream features such as estimated reach length, estimated stream width, sampling reach area, estimated stream depth, velocity, canopy cover, high water mark, proportion of reach represented by stream morphological types, channelization, and dam presence; • Surrounding woody debris in contact with the stream, noted by a wading visual observer; • The most dominant type of aquatic plants; • Water quality parameters such as temperature, conductivity, dissolved oxygen, pH, turbidity, water odors, water surface oils, and turbidity; and, • Sediment characteristics such as odors, oils, deposits, inorganic substrate components, and organic substrate components.	
Data Uses/Application	Evaluations of habitat quality via physical characteristics and water quality parameters are pertinent to any assessments of ecological integrity. These types of assessments are performed by many water resource agencies to determine if degraded habitat is the result of toxicity and/or pollution. The full assessment includes a general description of the site, the aforementioned physical characterization and water quality assessment, and a visual assessment of instream and riparian habitat quality (Fact Sheet 2.1.1-16).	
Advantages	Most of the aforementioned data can be collected with field investigations in a quick and relatively inexpensive manner.	
Limitations	These types of assessments are not sufficiently comprehensive to adequately identify all causes of impact.	
Reference	Barbour, et al., 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/owow/monitoring/rbp/ch05main.html#Section%205.2	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-16
Method Title	Visual-Based Habitat Assessment
Purpose	These methods describe the process for performing a visual-based habitat assessment of a stream. Habitat parameters pertinent to the assessment of habitat quality include those that characterize the stream "micro scale" habitat (*e.g.*, estimation of embeddedness), the "macro scale" features (*e.g.*, channel morphology) and the riparian and bank structure features that are most often influential in affecting the other parameters.
Method Summary	First, a 100 meter reach of a stream must be selected for the assessment. The entire sampling reach is then evaluated for each of the following parameters listed below: • Epifaunal substrate/available cover; • Embeddedness of rocks and snags; • Pool substrate characterization; • Velocity/depth combinations; • Pool variability; • Sediment deposition; • Channel flow status; • Channel alteration; • Frequency of riffles (or bends); • Channel sinuosity; • Bank stability; • Bank vegetative protection; and • Riparian vegetative zone width. An additional 7 general physical habitat attributes are also important in determining stream ecology: • Channel dimensions; • Channel gradient; • Channel substrate size and type; • Habitat complexity and cover; • Riparian vegetation cover and structure; • Anthropogenic alterations; and • Channel-riparian interaction. The habitat assessment process involves rating the parameters as optimal, suboptimal, marginal, or poor based on criteria included in the data sheets.
Data Uses/Application	Habitat assessments based on visual observation can be separated into 2 basic approaches- one designed for high-gradient streams and one designed for low-gradient streams. Some state programs have adapted this approach using somewhat different or fewer parameters.
Advantages	Standardized parameters list and protocol allows for some intercomparison amongst sites.
Limitations	Many of the data parameters are qualitative, thus assessments made at the same location by different biologists may vary. The protocol suggests that a team of 2 or more trained biologists should perform the assessment to enhance data quality.

Fact Sheet No.	2.1.1-16 (contd.)	
Reference	Barbour, et al., 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/owow/monitoring/rbp/ch05main.html#Section%205.2	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-17	
Method Title	USGS Field Operation Plan: Tributary Monitoring, in LMMB 017	
Purpose	This method describes the collection of water samples and field data from streams.	
Method Summary	At each proposed sampling station, a cross-section of the stream is measured, and the data will be used to subdivide the cross-section into three equal flow areas. The centroid of each of these areas is identified on a field map. At each centroid, water samples and Hydrolab parameters (*i.e.,* temperature, conductivity, dissolved oxygen, pH) are collected at 0.2 and 0.8 times the depth. Samples are collected during downstream flow, which is established for at least ½ hour prior to initiating sample collection. Water samples from each of the 6 sampling locations are composited. Water is collected for PCB, PAH, pesticide, and Atrazine analyses using a submersible pump and passed through a 293 mm, stainless steel, pentaplate filter holder. 2 - 5 glass fiber filters are used, depending on the concentration of suspended material in the water column. Filters will be folded and placed in aluminum foil pouches. The filtered samples will be stored in carboys until analysis. Water for DOC, POC, and conventional constituents is also collected using the pump. Secchi disk measurements are made at each centroid location for each cross-section. Velocity and flow direction are recorded at each subsampling location. Several ASTM Methods deal with the measurement of open channel flow. These methods include D1941, D3858, D4409, D5089, D5129, and D5130 (ASTM, 2001a).	
Data Uses/Application	Situations where samples are required for dissolved and organic contaminants.	
Advantages	This method provides a standardized approach for obtaining representative stream samples.	
Limitations	This collection method is limited to organic contaminants. Not applicable for trace metal contaminants. Not applicable for chlorophyll, dissolved/particulate nutrients, or TSS.	
Reference	USEPA. 1997b . Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/field96.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-18	
Method Title	Quality Assurance Plan for Discharge Measurements Using Broadband Acoustic Doppler Current Profilers	
Purpose	To measure velocities and discharge in the riverine and estuarine environment.	
Method Summary	The Acoustic Doppler Current Profiler (ADCP) is an electronic instrument that is used to measure water velocities. The instrument functions by transmitting acoustic signals into the water column. The velocity of particles in the water column, and therefore the velocity of the water, is calculated by comparing the frequency of the transmitted signals compared to the frequency of backscatter signals reflected off the particles. The instrument can be mounted to the side of a boat and towed through the water column. The ADCP measures the velocity of the water column relative to the movement of the vessel to which it is attached. Multiple transects of data are averaged to reduce variation due to turbulence and velocity surges. At least 4 transects should be made at each site to ensure a valid determination of discharge. Additional information regarding the use of ADCPs is found in Dredging Research Technical Note DRP-1-16 (U.S. Army Engineer Waterways Experiment Station 1994).	
Data Uses/Application	The ADCP can be used to measure water velocities and discharge in a variety of aquatic environments.	
Advantages	Measurement time is reduced; data can be collected throughout the water column and cross section; stationing devices are not necessary.	
Limitations	The ADCP and its associated software are complex systems that should be used only be highly trained personnel. High initial cost is also a major disadvantage. This instrumentation is also unable to function in shallow water. The ADCP can accurately measure discharge for only a limited range of flow conditions.	
Reference	Lipscomb, SW. 1995. Quality Assurance Plan for Discharge Measurements Using Broadband Acoustic Doppler Current Profilers. USGS Open-File Report 95-701.	
Website	http://il.water.usgs.gov/adcp/reports/OFR95-701.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-19	
Method Title	Seepage Meters	
Purpose	To determine the exchange of ground water to surface water and vice versa.	
Method Summary	The Krupaseep is a prototype seepage meter device constructed of translucent polycarbonate plastic domes of varying size. Each dome has a vertical skirt to anchor it into deep organic muds. A port in the top of each dome allows flow-through of water. Flow meters are installed in this port, and water quality meters are installed on the inside and outside of the dome. When in use, the domes are pressed into the river bottom until the top of each dome is 14 inches above the mudline. The monitoring equipment on each dome is tethered back to computers onshore that record real-time water quality data, including photosynthesis-activated radiation, on the inside and outside of the seepage meter and record the inflow or outflow (flux) via heat pulse technology. Riverside solar panels charge the batteries that power the flow meter computer and data logger. Water quality samples can also be collected using hoses mounted on both the internal and external surfaces of the dome and a onshore peristaltic pump.	
Data Uses/Application	A knowledge of the groundwater flux is needed to evaluate the significance of contaminant flux into the water column from capped contaminated sediments.	
Advantages	This unit allows for the collection, both continuous and discrete, of water quality parameters both inside and outside of the seepage meter. Measures of flux and water quality can be made remotely.	
Limitations	The location of the meter is restricted to a radial operating distance of 160 feet from the computers. Divers are required for installation and service of the units. Weekly maintenance is required under harsh environmental conditions.	
Reference	USGS. 2001. The Krupaseep. Next Generation Seepage Meter.	
Website	http://sofia.usgs.gov/sfrsf/entdisplays/krupaseep/	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.1-20	
Method Title	Caged Bivalve Deployment, AED LOP 1.02.002, Revision 1	
Purpose	Deployment and retrieval of caged bivalves for environmental monitoring.	
Method Summary	Four mussel baskets are deployed along a trawl (similar to long-line). Each trawl has a surface floatation (lobster buoy) and an anchor (cinder block) at either end. The mussel baskets are attached by leaders to cinder blocks fixed along the trawl and are suspended approximately one meter above the bottom with sub-surface floatation. Four replicate mussel baskets placed 50' apart are adequate to characterize an area for chemical bioaccumulation, though this number dependent upon localized conditions. The desired length of the trawl line is 170' plus 2X the high water depth at the deployment location.. Each basket is fabricated from a 18" x 12" rectangle of black polyethylene ½" mesh netting rolled into a cylinder, and secured with cable ties. The ends of the cylinder are also closed with cable ties. Each basket contains 25 blue mussels, 5-7 cm in length. The baskets are prepare in the laboratory and the baskets and mussels are transported to the field site in coolers. In the field, the mussel baskets are attached to the trawl leaders with cable ties. The trawl is deployed sequentially beginning with one surface buoy followed by each of the cinder blocks and finally the second surface buoy. Retrieval is the reverse of deployment, beginning with the “down wind” buoy. Mussel baskets should be placed in coolers as they are removed from the line.	
Data Uses/Application	Caged bivalve deployments are used to obtain tissue for chemical bioaccumulation studies, frequently as part of environmental monitoring projects.	
Advantages	Relatively low cost and simple method with potential for revealing different biomarkers of toxicity, identifying the chemicals responsible for the effects measured, delineating affected areas, and specifying risks to aquatic fauna of environmental contamination.	
Limitations	Permits may be required from local harbor master and/or appropriate regulatory agencies. Other users of waterway, such as boat captains ans commercial lobster men, must be considered. Weather may inhibit or prohibit deployment and retrieval. This procedure was written to meet the specific needs of the research program at the U.S. EPA-Atlantic Ecology Division. It is not a U.S. EPA Standard Method and must not be referred to as such. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.	
Reference	Unpublished laboratory SOP, EPA NHEERL-AED, Narragansett, RI	
Website	N/A	Last Accessed:

2.1.2 Chemical and Physical Analyses

Section 2.1.2 contains methods for sample preparation and chemical and physical analysis of water. These methods characterize the chemical composition and physical properties of water samples collected by methods described in Section 2.1.1. Samples are often analyzed for the presence of various inorganic and organic contaminants that may pose a threat to human or ecological health. Many of the methods described have been developed over time to optimize the detection, identification, and quantification of potential chemicals of concern. Several are performance-based and may be further modified to enhance the accuracy and precision of the method.

A variety of methods may exist for the analysis of a particular chemical parameter, all with varying levels of quantification or degrees of sensitivity. Less sensitive methods may be used as a screening tool during the initial site assessment to identify potential chemicals of concern. Follow-up analysis may include the use of very precise methods that provide unequivocal identification and trace level quantification of analytes. This variety also provides alternative methods useful in the analysis of many types of water samples. Interferences from certain compounds in a water sample may be avoided by the use of an alternative method.

Other than describing the water column itself, the physical properties of water often influence the behavior of contaminants in the water column, and they may be helpful in further understanding the fate of contaminants in the environment. Physical parameters may change the solubility and chemical form of various chemical components in water.

Many of the chemical and physical methods described in these fact sheets are routinely performed and fairly standardized. As a result, more than one source of information is often cited in each method description. Specifically, the following sources provided methods information for section 2.1.2:

- The USEPA's Office of Water
- The USEPA's Lake Michigan Mass Balance Study Methods Compendium, 1997v The USEPA's Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (SW846 Methods)
- NOAA's National Status and Trends Program, 1998
- Standard Methods for Examination of Water and Wastewater, 1999
- ASTM

Fact Sheet No.	2.1.2-1	
Method Title	Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, EPA Method 245.7	
Purpose	To determine the concentration of mercury (Hg) in filtered and unfiltered water by cold-vapor atomic fluorescence spectrometry (CVAFS).	
Method Summary	A 100- to 2000-mL sample is collected directly into a fluoropolymer bottle using sample handling techniques specially designed for collection of mercury at trace levels. For dissolved Hg, the sample is filtered through a 0.45-µm capsule filter. The sample is preserved by adding 5 mL/L of pretested 12N HCl. Inorganic Hg compounds and organic mercury species are oxidized by a potassium bromate/potassium bromide reagent. After oxidation, the sample is sequentially prereduced with $NH_2OH{\cdot}HCl$ to destroy the excess bromine, then the ionic Hg is reduced with $SnCl_2$ to convert Hg(II) to volatile Hg(0). The Hg(0) is separated from solution by purging with high purity argon gas through a semipermeable dryer tube. The Hg passes into an inert gas stream that carries the released Hg(0) into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection. The concentration of Hg is determined by atomic fluorescence spectrometry at 253.7 nm. The method detection limit (MDL) and minimum level of quantization (ML) in this method are 1.8 ng/L and 5.0 ng/L, respectively. This method may be used to determine Hg up to 200 ng/L and may be extended by dilution of the sample. The normal calibration range for ambient water monitoring is 5 ng/L to 100 ng/L. A similar method for the detection of total mercury is ASTM Method D3223 (ASTM, 2001a).	
Data Uses/Application	This method is applicable to drinking water, surface and ground waters, marine water, and industrial and municipal wastewater.	
Advantages	Wide analytical range makes method suitable for contaminated sites.	
Limitations	Ambient mercury levels frequently are below the detection limit provided by this method.	
Reference	USEPA. 2001a. Method 245.7: Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Draft, EPA 821-R-01-008. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.1.2-2
Method Title	Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, EPA Method 1631, Revision B
Purpose	This method is for determination of mercury (Hg) in filtered and unfiltered water by oxidation, purge and trap, desorption, and cold-vapor atomic fluorescence spectrometry (CVAFS).
Method Summary	A 100- to 2000-mL sample is collected directly into a fluoropolymer bottle. For dissolved Hg, the sample is filtered through a 0.45-µm capsule filter. The sample is preserved by adding either HCl or BrCl solution. If a sample will also be used for the determination of methyl mercury, it should be preserved with HCl solution only. Prior to analysis, a 100-mL sample aliquot is placed in a specially designed purge vessel, and 0.2N BrCl solution is added to oxidize all Hg compounds to Hg(II). After oxidation, the sample is sequentially prereduced with $NH_2OH{\cdot}HCl$ to destroy the free halogens, then reduced with $SnCl_2$ to convert Hg(II) to volatile Hg(0). The Hg(0) is separated from solution by purging with nitrogen onto a gold-coated sand trap. The trapped Hg is thermally desorbed from the gold trap and carried into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection. This method is for determination of Hg in the range of 0.5–100 ng/L and may be extended to higher levels by selection of a smaller sample size. The method detection limit for Hg has been determined to be 0.2 ng/L when no interferences are present. The minimum level of quantization (ML) has been established as 0.5 ng/L. An MDL as low as 0.05 ng/L can be achieved for low Hg samples by using a larger sample volume, a lower BrCl level (0.2%), and extra caution in sample handling. For the analysis of water samples using Methods LMMB 048 and 049 (USEPA, 1997d), subsamples are oxidized with BrCl solution and heated for at least an hour (preferably overnight) at 70°C before prereduction and analysis. Aliquots of 125-500 mL are purged and trapped. For particulate samples, the filter is treated in a similar fashion as the water samples, except that 2 mL of hydroxylamine HCl is used to produce samples. Method LMMB 048 has a mean detection limit of approximately 0.1 ng/L.
Data Uses/Application	This method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.
Advantages	The dual amalgam trap system and fluorescence detector provide greater sensitivity and specificity in the presence of interferences, and this system must be used to overcome interferences, if present, and to achieve the sensitivity required, if necessary. The detection range of this method generally provides detection of mercury in ambient surface waters.
Limitations	This method does not distinguish between methyl mercury and inorganic mercury species.

Fact Sheet No.	2.1.2-2 (contd.)	
Reference	USEPA. 1999b. Method 1631, Revision B: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, EPA 821-R-99-005. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-3	
Method Title	Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS, EPA Method 1630	
Purpose	This method is designed for determination of CH_3Hg in the range of 0.02-5 ng/L and may be extended to higher levels by selection of a smaller sample size.	
Method Summary	A 100-2000 mL sample is collected directly into fluoropolymer or borosilicate bottle(s). For dissolved CH_3Hg, samples are filtered through a 0.45-µm capsule filter. Fresh water samples are preserved by adding 11.6 M HCl, while saline samples ([Cl -] > 500 ppm) are preserved with 9 M H_2SO_4. Prior to analysis, a 45-mL sample aliquot is placed in a specially designed fluoropolymer distillation vessel, and 35 mL of the water is distilled into the receiving vessel at 125° C under N_2 flow. After distillation, the sample is adjusted to pH 4.9 and ethylated in a closed purge vessel. The ethyl analog of CH_3Hg, methylethyl mercury, is separated from solution by purging with N_2 onto a graphitic carbon (Carbotrap ®) trap. The trapped methylethyl mercury is thermally desorbed from the trap, carried through a pyrolytic decomposition column, which converts organo mercury forms to elemental mercury (Hg 0), and then into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection. The method detection limit for CH_3Hg has been determined to be 0.02 ng/L when no background elements or interferences are present. The minimum level (ML) has been established as 0.06 ng/L. An MDL as low as 0.009 ng/L can be achieved for low CH_3Hg samples by using extra caution in sample handling and reagent selection, particularly the use of “for ultra-low level only” distillation equipment.	
Data Uses/Application	This method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.	
Advantages	Methyl mercury is frequently required for risk assessments. This method provides the sensitivity to determine ambient levels in most aqueous samples.	
Limitations	Samples may become contaminated by numerous routes. Potential sources of trace metals contamination include: metallic or metal-containing labware (*e.g.*, talc gloves that contain high levels of zinc), containers, sampling equipment, reagents, and reagent water; improperly cleaned or stored equipment, labware, and reagents; and atmospheric inputs such as dirt and dust. Even human contact can be a source of trace metals contamination.	
Reference	USEPA. 2001b. Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS, EPA 821-R-01-020. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.1.2-4
Method Title	Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption, EPA Method 1639
Purpose	This procedure is for the determination of dissolved elements (antimony, cadmium, nickel, selenium, trivalent chromium, zinc) in ambient waters by Stabilized Temperature Graphite Furnace Atomic Absorption (GFAA). It may also be used for determination of total recoverable element concentrations in these waters.
Method Summary	For total recoverable analysis of an aqueous sample containing undissolved material, analytes are first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample is made up to volume, mixed, and centrifuged or allowed to settle overnight prior to analysis. To determine dissolved analytes in a filtered aqueous sample aliquot, the sample is prepared for analysis by the appropriate addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis. The analytes listed in this method are determined by stabilized temperature platform graphite furnace atomic absorption (STPGFAA). **Metal** — **MDL (µg/L)** — **ML (µg/L)** Antimony — 1.9 — 5 Cadmium — 0.023 — 0.05 Chromium (III) — 0.1 — 0.2 Nickel — 0.65 — 2 Selenium — 0.83 — 2 Zinc — 0.14 — 0.5 ASTM Method D1687 describes a similar (and alternative) method for the measurement of hexavalent and total chromium in water (ASTM, 2001a). ASTM Methods D3557 and D3859 describe the analysis of cadmium and selenium by GFAA, respectively. ASTM Method D3919 and SW846 Method 7000A describe the analysis of several elements by atomic absorption methods (ASTM, 2001a; USEPA SW846.
Data Uses/Application	This method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.
Advantages	GFAA techniques in addition to ICP-MS methods are frequently required to obtain all trace metal analytes of interest.
Limitations	Samples may become contaminated by numerous routes. This method should be used by analysts experienced in the use of graphite furnace atomic absorption spectroscopy.
Reference	USEPA. 1996b. Method 1639: Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption, EPA 821-R-96-006. Office of Water, U.S. Environmental Protection Agency, Washington, DC.
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html — Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-5	
Method Title	Determination of Trace Elements in Ambient Waters by Off-Line Chelation Pre-concentration and Stabilized Temperature Graphite Furnace Atomic Absorption, EPA Method 1637	
Purpose	This procedure is for the determination of dissolved elements in ambient waters, namely cadmium and lead. It may also be used for determination of total recoverable element concentrations in these waters.	
Method Summary	For total recoverable analysis of an aqueous sample containing undissolved material, analytes are first solubilized with nitric acid. After cooling, the sample is made up to volume, mixed, and centrifuged or allowed to settle overnight before analysis. For the determination of dissolved analytes in a filtered aqueous sample aliquot, the sample is made ready for analysis by the appropriate addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis. This method is used to preconcentrate trace elements using an iminodiacetate functionalized chelating resin. After a sample is prepared, it is buffered using an on line system before it enters the chelating column. Group I and II metals, as well as most anions, are selectively separated from the analytes by elution with ammonium acetate at pH 5.5. The analytes are subsequently eluded into a simplified matrix consisting of 0.75 M nitric acid. The eluded sample is collected and then analyzed by stabilized temperature platform graphite furnace atomic absorption (STPGFAA). The method detection limits for Cd and Pb have been determined to be 0.0075 µg/L and 0.036 µg/L, respectively. The minimum levels (ML) have been established as 0.02 and 0.1 µg/L, respectively. Similar methods include ASTM Methods D3557 for cadmium and D3559 for lead (ASTM, 2001a).	
Data Uses/Application	This method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.	
Advantages	Off line Pre-concentration methods eliminate many sample handling procedures, reducing sources of contamination. GFAA analysis affords extremely low detection limits.	
Limitations	Due to its sensitivity, interferences can occur with GFAA analysis. This method should be used by analysts experienced in the use of graphite furnace atomic absorption spectroscopy.	
Reference	USEPA. 1996c. Method 1637: Determination of Trace Elements in Ambient Waters by Off-Line Chelation Pre-concentration and Stabilized Temperature Graphite Furnace Atomic Absorption, EPA 821-R-96-004. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-6
Method Title	Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma — Mass Spectrometry, EPA Method 1638
Purpose	This procedure is for the determination of dissolved elements (antimony, cadmium, copper, lead, nickel, selenium, silver, thallium, zinc) in ambient waters. It may also be used for determination of total recoverable element concentrations in these waters.
Method Summary	For total recoverable analysis of an aqueous sample containing undissolved material, analytes are first solubilized with nitric and hydrochloric acids. After cooling, the sample is made to volume, mixed, and centrifuged or allowed to settle overnight prior to analysis. For the determination of dissolved analytes in a filtered aqueous sample aliquot, the sample is prepared for analysis by the appropriate addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis. The digested sample is introduced into a radio frequency plasma, where energy transfer processes cause desolvation, atomization, and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to- charge ratio (m/z) by a mass spectrometer. Ions transmitted through the mass analyzer are detected by an electron multiplier or Faraday detector and the resulting current is processed by a data handling system.

Metal	1638 MDL (µg/L)	1638 ML (µg/L)	LMMB 057 ML (ng/L)
Aluminum			25/15
Antimony	0.0097	0.02	
Arsenic			15/10
Cadmium	0.025		2.5/0.5
Chromium			20/8
Copper	0.087	0.2	8/4
Lead	0.015	0.05	3/0.5
Nickel	0.33	1	
Selenium	0.45	1	
Silver	0.029	0.1	1.5/0.3
Thallium	0.0079	0.02	
Zinc	0.14	0.5	10/2.5

ICP-MS detection limits listed for LMMB 057 are for pneumatic and ultrasonic nebulization, respectively (USEPA, 1997c).

Standard Method 3120B, ASTM Method D5673, and SW846 Method 6020 describe the analysis of metals by ICP-MS (APHA, 1999; ASTM, 2001a; USEPA SW846).

Data Uses/Application	This method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.

Fact Sheet No.	2.1.2-6 (contd.)	
Advantages	Analysis by ICP-MS provides a high level of sensitivity for some elements that are difficult to determine by other methods. Up to 20 elements can be determined from a single sample.	
Limitations	This method should be used by analysts experienced in the use of inductively coupled plasma mass spectrometry (ICP-MS).	
Reference	USEPA. 1996d. Method 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma-Mass Spectrometry, EPA 821-R-96-005. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-7	
Method Title	Determination of Trace Elements in Ambient Waters by On-Line Chelation Pre-concentration and Inductively Coupled Plasma-Mass Spectrometry, EPA Method 1640	
Purpose	This procedure is for the determination of dissolved elements (cadmium, copper, lead, nickel) in ambient waters. It may also be used for determination of total recoverable element concentrations in these waters.	
Method Summary	This method is used to preconcentrate trace elements using an iminodiacetate functionalized chelating resin system that is connected directly to the ICP-MS. Following acid solubilization, the sample is buffered prior to the chelating column using an on line system. Group I and II metals, as well as most anions, are selectively separated from the analytes by elution with ammonium acetate at pH 5.5. The analytes are subsequently eluded into a simplified matrix consisting of dilute nitric acid and are determined by ICP-MS using a directly coupled on line configuration. **Metal** **MDL (µg/L)** **ML (µg/L)** Cadmium 0.0024 0.01 Copper 0.024 0.1 Lead 0.0081 0.02 Nickel 0.029 0.1	
Data Uses/Application	This method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.	
Advantages	The on line Pre-concentration system allows for reduced sampling handling, minimizing the risk of sample contamination. Method 1640 is a convenient method for the detection of a short list of toxic metals.	
Limitations	Neither mercury nor arsenic can be measured with this method.	
Reference	USEPA. 1996e. Method 1640: Determination of Trace Elements in Ambient Waters by On-Line Chelation Pre-concentration and Inductively Coupled Plasma-Mass Spectrometry, EPA 821-R-96-007. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-8	
Method Title	Inorganic Arsenic in Water by Hydride Generation Quartz Furnace Atomic Absorption, EPA Method 1632	
Purpose	This method is for determination of total inorganic arsenic (As) in filtered and unfiltered water by hydride generation and quartz furnace atomic absorption detection. This method is designed for measurement of dissolved and total arsenic in the range of 10-200 ng/L.	
Method Summary	A 100-2000 mL sample is collected directly into a sample bottle. The sample is either field or laboratory preserved by the addition of 10% HNO_3, depending on the time between sample collection and arrival at the laboratory. An aliquot of sample is placed in a specially designed reaction vessel and 6 M HCl is added. Before analysis, 4% $NaBH_4$ solution is added to convert organic and inorganic arsenic to volatile arsines. The arsines are purged from the sample onto a cooled glass trap packed with 15% OV-3 on Chromasorb ® WAW-DMCS0, or equivalent. The trapped arsines are thermally desorbed, in order of increasing boiling points, and carried into the quartz furnace of an atomic absorption spectrophotometer for detection. The first arsine to be desorbed will be AsH_3 , which represents total inorganic arsenic in the sample. The method detection limit for total inorganic arsenic has been determined to be 3 ng/L when no background elements or interferences are present. The minimum level (ML) has been established at 10 ng/L. ASTM Method D2972B, Standard Method 3114B, and SW846 Method 7061A all describe similar methods for the hydride generation atomic absorption detection of arsenic in water (ASTM, 2001a; APHA, 1999; USEPA SW 846)	
Data Uses/Application	The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act.	
Advantages	Automation of this method reduces error and potential sources of contamination.	
Limitations	This method does not provide data on arsenic speciation, which is sometimes required in risk assessments.	
Reference	USEPA. 1996f. Method 1632: Inorganic Arsenic in Water by Hydride Generation Quartz Furnace Atomic Absorption, EPA 821-R-96-013. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-9	
Method Title	Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry, EPA Method 1632, Revision A	
Purpose	This method is for determination of inorganic arsenic (IA), arsenite (As +3), arsenate (As +5), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) in filtered and unfiltered water by hydride generation and quartz furnace atomic absorption detection. This method is designed for measurement of As species in water in the range 0.01-50 µg/L.	
Method Summary	Aqueous sample—A 500- to 1000-mL water sample is collected directly into a cleaned sample bottle. Water samples are preserved in the field by the addition of 6M HCl. The recommended holding time is 28 days. An aliquot of water sample is placed in a specially designed reaction vessel, and 6M HCl is added. $NaBH_4$ solution is added to convert IA, MMA, and DMA to volatile arsines. Arsines are purged from the sample onto a cooled glass trap packed with 15% OV-3 on Chromosorb ® W AW-DMCS, or equivalent. The trapped arsines are thermally desorbed, in order of increasing boiling points and carried into the quartz furnace of an atomic absorption spectrophotometer for detection. To determine the concentration of As +3, another aliquot of water sample or tissue digestate is placed in the reaction vessel and Tris-buffer is added. The procedure is repeated to quantify only the arsine produced from As +3. The concentration of As +5 is the concentration of As +3 subtracted from the concentration of IA. **Analyte** **MDL** **ML** IA (As +3 +As +5) 0.003 µg/L 0.01 µg/L Arsenite (As +3) 0.003 µg/L 0.01 µg/L MMA 0.004 µg/L 0.01 µg/L DMA 0.02 µg/L 0.05 µg/L	
Data Uses/Application	The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act.	
Advantages	The relative amounts of carcinogenic arsenite (As +3) to total arsenic varies with surface water body and varies with pH. This method directly quantifies arsenite.	
Limitations	Depending upon As levels at site, speciation may not be necessary.	
Reference	USEPA. 2001c. Method 1632, Revision A: Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry, EPA 821-R-01-006. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.1.2-10	
Method Title	Determination of Hexavalent Chromium by Ion Chromatography, EPA Method 1636	
Purpose	This method is for the determination of dissolved hexavalent chromium (as CrO_4^{2-}) in ambient waters at EPA water quality criteria (WQC) levels using ion chromatography (IC).	
Method Summary	An aqueous sample is filtered through a 0.45 µm filter, and the filtrate is adjusted to a pH of 9-9.5 with a concentrated buffer solution. A measured volume of the sample (50-250 µL) is introduced into the ion chromatography. A guard column removes organics from the sample before the Cr(VI), as CrO42-, is separated on a high capacity anion exchange separator column. Post column derivatization of the Cr(VI) with diphenylcarbazide is followed by detection of the colored complex at 530 nm. The method detection limit (MDL), and the minimum level (ML) for hexavalent chromium (Cr(VI)) are 0.23 µg/L and 0.5 µg/L, respectively. ASTM Method D5257 and SW846 Method 7199 also describe the analysis of hexavalent chromium by Ion Chromatography (ASTM, 2001a; USEPA SW846).	
Data Uses/Application	The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act.	
Advantages	This method provides the rapid and reproducible isolation and analysis of Cr (VI) without interference from other Cr species.	
Limitations	Samples containing high levels of anionic species, such as sulfate and chloride, may cause column overload. Samples containing high levels of organics or sulfides cause rapid reduction of soluble Cr(VI) to Cr(III). Samples must be stored at 4°C and analyzed within 24 hours of collection unless preserved with sodium hydroxide.	
Reference	USEPA. 1996g. Method 1636: Determination of Hexavalent Chromium by Ion Chromatography, EPA 821-R-95-029. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html	Last Accessed: 2/12/03

Fact Sheet No.	2.1.2-11	
Method Title	Volatile Organic Compounds by Isotope Dilution GC/MS, EPA Method 1624b	
Purpose	This method is designed to determine the volatile toxic organic pollutants.	
Method Summary	Stable isotopically labeled analogs of the compounds of interest are added to a 5 mL water sample. The sample is purged at 20-25˚C with an inert gas in a specially designed chamber. The volatile organic compounds are transferred from the aqueous phase into the gaseous phase where they are passed into a sorbent column and trapped. After purging is completed, the trap is back flushed and heated rapidly to desorb the compounds into a gas chromatography (GC). The compounds are separated by the GC and detected by a mass spectrometer (MS). The labeled compounds serve to correct the variability of the analytical technique. Identification of a compound (qualitative analysis) is performed by comparing the GC retention time and the background corrected characteristic spectral masses with those of authentic standards. Quantitative analysis is performed by GC/MS using extracted ion current profile (EICP) areas. Isotope dilution is used when labeled compounds are available; otherwise, an internal standard method is used. The Minimum Level for most VOC compounds is either 10 or 50 µg/L. Similar methods for the determination of volatile organic compounds and volatile aromatic organic compounds are presented in Standard Methods 6210 and 6220, respectively (APHA, 1999). SW846 Methods 5030B, 5035, and 8260B and ASTM Method D5790 describe the preparation and analysis of volatile organics by GC/MS (USEPA SW846); ASTM, 2001a).	
Data Uses/Application	The method is designed to meet the survey requirements of Effluent Guidelines Division (EGD) and the National Pollutants Discharge Elimination System (NPDES) under 40 CFR Parts 136.1 and 136.5. VOC in ambient waters is sometimes monitored at sites of suspected contaminated groundwater inflow.	
Advantages	The combination of GC retention time and MS characterization provides unequivocal identification of analytes.	
Limitations	The GC/MS portions of this method are for use only by analysts experienced with GC/MS or under the close supervision of such qualified persons. Samples can be contaminated by diffusion of volatile organic compounds (particularly methylene chloride) through the bottle seal during shipment and storage. Contamination by carry-over can occur.	
Reference	USEPA. 1989a. Method 1624, Revision B: Volatile Organic Compounds by Isotope Dilution GC/MS, EPA440-1-89-023. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	Rev B not available online	Last Accessed:

Fact Sheet No.	2.1.2-12	
Method Title	Photovac GC Analysis for Soil, Water, and Air/Soil Gas, ERT SOP# 2109	
Purpose	This method is designed as a field screening procedure for the tentative identification of various volatile organic compounds.	
Method Summary	Water samples are collected in triplicate in 40-mL VOA vials with Teflon™-lined silicone septum screw caps. The vials are filled completely, with no visible air bubbles. A 20-mL aliquot of sample from one of the three sample triplicates is pipetted into a second, clean VOA vial. The vial is capped, shaken vigorously for one minute, and allowed to stand at room temperature for at least 30 minutes for vapor phase equilibration. An aliquot of the water head space is then removed from the vial and injected into the GC using a gas-tight syringe. The GC uses an ultraviolet light source and photoionization detector. The other two vials are analyzed within seven days by another method to confirm the field screening data. Typical MDLs for this method range from 1 ppb to 5 ppb. ERT SOPs # 2108 and #2107 describe the operation of specific models of Photovac Gas Chromatographs.	
Data Uses/Application	Site assessment/characterization and health and safety surveys.	
Advantages	The data generated with this method allows for rapid evaluation of site conditions.	
Limitations	Pollutant identification is only tentative.	
Reference	USEPA. 1994b. SOP # 2109: Photovac GC Analysis for Soil, Water, and Air/Soil Gas. Environmental Response Team. Compendium of ERT Standard Operating Protocols. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison NJ.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.1.2-13	
Method Title	Semi-volatile Organic Compounds by Isotope Dilution GC/MS, EPA Method 1625 Revision B	
Purpose	This method is used to determine the semi-volatile toxic organic pollutants (*i.e.,* PAHs) in water.	
Method Summary	This method is performance-based. Stable isotopically labeled analogs of the compounds of interest are added to a one liter water sample. The sample is extracted at pH 12-13, then at pH <2 with methylene chloride using continuous extraction techniques. The extract is dried over sodium sulfate and concentrated to a volume of 1 mL. An internal standard is added to the extract, and the extract is injected into the gas chromatography (GC). The compounds are separated by GC and detected by a mass spectrometer (MS). The labeled compounds serve to correct the variability of the analytical technique. Identification of a compound (qualitative analysis) is performed by comparing the GC retention time and background corrected characteristic spectral masses with those of authentic standards. Quantitative analysis is performed by GC/MS using extracted ion current profile (EICP) areas. Isotope dilution is used when labeled compounds are available; otherwise, an internal standard method is used. The method detection limit for most compounds of interest is 10 ug/L. The MDL for some compounds is 20 or 50 ug/L. Similar methods for the extraction and analysis of semi-volatile organic compounds are Standard Method 6410B and SW846 Method 8270C (APHA, 1999).	
Data Uses/Application	The method is designed to meet the survey requirements of Effluent Guidelines Division (EGD) and the National Pollutants Discharge Elimination System (NPDES) under 40 CFR Part 136.1.	
Advantages	Mass spectral analysis, combined with gas chromatographic compound retention time, provides unequivocal compound identification. Isotope dilution corrects recovery and performance of each compound of interest.	
Limitations	The GC/MS portions of this method are for use only by analysts experienced with GC/MS or under the close supervision of such qualified persons. The suites of stable isotopes required for this analysis are often prohibitively expensive for use in routine monitoring programs.	
Reference	USEPA. 1989b. Method 1625, Revision B: Semi-volatile Organic Compounds by Isotope Dilution GC/MS, EPA440-1-89-023. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	Rev B not available online	Last Accessed:

Fact Sheet No.	2.1.2-14	
Method Title	Quantitative Determination of Polynuclear Aromatic Hydrocarbons by Gas Chromatography/Mass Spectrometry (GC/MS) - Selected Ion Monitoring (SIM) Mode	
Purpose	To determine low concentrations of polycyclic aromatic hydrocarbons (PAHs) and their alkylated homologues in extracts of water, sediments and biological tissues.	
Method Summary	Just prior to analysis, an aliquot of internal standard solution is added to the sample extract producing a final internal standard concentration of approximately 40 ng/mL. The analytical system includes a temperature programmable gas chromatography with a DB-5MS fused silica capillary column. Helium is used as the carrier gas, and the samples are handled by an auto sampler capable of making 1 - 4 µl injections. A five point calibration curve is established to demonstrate the linear range of the detector. The effluent from the GC capillary column is routed directly into the ion source of the mass spectrometer (MS). The MS is operated in the SIM mode using appropriate windows to include the quantization and confirmation masses for target PAHs. For all compounds detected at a concentration above the MDL, a confirmation ion is checked to confirm its presence. The response factors of the surrogate relative to each of the calibration standards are calculated, followed by the calculation of the sample extract concentration. The sample concentration for each compound is calculated by dividing the sample extract concentration by the sample amount.	
Data Uses/Application	PAH concentrations, particularly pyrene, are one of the primary risk factors associated with contaminated waters. PAH data obtained from this analysis are used for site characterization and risk assessments.	
Advantages	GC/MS in the SIM mode provides unambiguous and sensitive detection for PAHs. The PAH quantization method is very rigorous because PAHs have very strong molecular ion peaks under the mass spectrometric conditions used. Also, the availability of labeled surrogates internal standards of many of the analytes makes very accurate determinations of analyte concentrations possible. Analysis of alkylated PAH homologues can provide site-specific information that can be used in source identification or product identification.	
Limitations	GC/MS in SIM mode cannot be used for simultaneous screening for other organic contaminants of similar polarity or volatility; cannot be used to identify tentatively identified compounds (TICs).	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.1.2-15	
Method Title	Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection, LMMB 041	
Purpose	To quantify chlorinated hydrocarbons (*i.e.,* chlorinated pesticides and PCBs) in sample extracts.	
Method Summary	This method is based on high resolution, capillary gas chromatography using electron capture detection (GC/ECD). Extracts normally have a holding time of 40 days. This method provides for initial, ongoing and final calibrations, which are done as part of the analytical run. If the response for any peak exceeds the highest calibration solution, the extract is diluted, a known amount of surrogate and TCMX solution added, and the sample reanalyzed for those analytes that exceeded the calibration range. Concentrations in the samples are calculated based on the internal standard method. Data are reported as ng/g dry weight. Other methods describing the analysis of PCBs and pesticides by GC/ECD are NS&T methods, ASTM Methods D5317 and D3534, and SW846 Methods 8081A and 8082 (NOAA, 1998; ASTM, 2001a).	
Data Uses/Application	PCBs and persistent pesticides (particularly DDT and metabolites) are two of the primary risk factors of contaminated waters. Data are used in site characterization and in risk analysis.	
Advantages	The ECD is very sensitive and allows for detection of the chlorinated hydrocarbons at trace concentrations (ppb).	
Limitations	The detector does not have a linear response over a wide concentration range and must be used by sufficiently trained personnel. Second column analysis must be performed to provide unequivocal compound identification. These methods do not measure the 12 World Health Organization congeners, which may be desired data in some risk assessments.	
Reference	USEPA. 1997d. Lake Michigan Mass Balance Study Methods Compendium, Volume 2: Organic and Mercury Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/sop-501.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-16	
Method Title	PCBs and Pesticides in Surface Water by XAD-2 Resin Extraction, LMMB 039	
Purpose	This method is used to determine congener specific PCB and pesticide concentrations at trace levels in surface water.	
Method Summary	Water samples (80 - 160 L) are filtered for particulates and the dissolved PCBs are collected using an XAD-2 resin column. Filters and resin columns are stored at 4°C until analysis. The filters and resin are Soxhlet extracted using 50% acetone/50% hexane for 16 hours. The water remaining in the samples is extracted with hexane. The sample extracts are concentrated and dried with sodium sulfate. The samples are run through a Florisil column and then through a silica column. Two fractions are collected from the silica column. The first fraction is eluded with hexane and contains the PCBs, HCB, and p,p' DDE. The second fraction is eluded with 25% ethyl ether in hexane and contains alpha-BHC, lindane, the chlordanes, nonachlors, p,p'DDD, p,p'DDT, and toxaphene. These fractions are concentrated and further cleaned with sulfuric acid. Sample extracts are analyzed by GC/ECD according to Method LMMB 041. Confirmation of pesticides in the second fraction is performed on a second column or by GC/MS. Preparation of XAD-2 Resin is presented in SW846 Method 0010: Appendix A (USEPA SW846).	
Data Uses/Application	Water quality assessments under Clean Water Act, RCRA, or CERCLA	
Advantages	Extraction of water through resin permits the economical extraction of large volumes (>20 L) with this method.	
Limitations	XAD-Z resin is difficult to prepare for trace PCB application.	
Reference	USEPA. 1997d. Method LMMB 039: PCBs and Pesticides in Surface Water by XAD-2 Resin Extraction, Lake Michigan Mass Balance Study Methods Compendium, Volume 2: Organic and Mercury Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/sec1293.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-17
Method Title	Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, EPA Method 1613
Purpose	This method is for determination of tetra through octa-chlorinated dibenzo-p-dioxins (CDDs) and dibenzo furans (CDFs) in water.
Method Summary	This method is "performance-based." Stable isotopically labeled analogs of 15 of the 2,3,7,8-substituted CDDs/CDFs are spiked into a 1 L water sample, and the sample is extracted by one of three procedures: 1. Samples containing no visible particles are extracted with methylene chloride in a separatory funnel or by the solid-phase extraction technique. The extract is concentrated for cleanup. 2. Samples containing visible particles are vacuum filtered through a glass-fiber filter. The filter is extracted with toluene in a Soxhlet/Dean-Stark (SDS) extractor, and the filtrate is extracted with methylene chloride in a separatory funnel. The methylene chloride extract is concentrated and combined with the SDS extract prior to cleanup. 3. The sample is vacuum filtered through a glass-fiber filter on top of a solid-phase extraction (SPE) disk. The filter and disk are extracted in an SDS extractor, and the extract is concentrated for cleanup. After extraction, $_{37}Cl_4$-labeled 2,3,7,8-TCDD is added to each extract to measure the efficiency of the cleanup process. Sample cleanups may include back-extraction with acid and/or base, and gel permeation, alumina, silica gel, Florisil and activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of the 2,3,7,8-isomers or other specific isomers or congeners. After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, internal standards are added to each extract, and an aliquot of the extract is injected into the gas chromatography. The analytes are separated by the GC and detected by a high-resolution ($\geq$10,000) mass spectrometer. **CDD/CDF** — **Minimum Level (pg/L)** — **CDD/CDF** — **ML(pg/L)** 2,3,7,8-TCDF — 10 — 1,2,3,4,7,8-HxCDD — 50 2,3,7,8-TCDD — 10 — 1,2,3,6,7,8-HxCDD — 50 1,2,3,7,8-PeCDF — 50 — 1,2,3,7,8,9-HxCDD — 50 2,3,4,7,8-PeCDF — 50 — 1,2,3,4,6,7,8-HpCDF — 50 1,2,3,7,8-PeCDD — 50 — 1,2,3,4,7,8,9-HpCDF — 50 1,2,3,4,7,8-HxCDF — 50 — 1,2,3,4,6,7,8-HpCDD — 50 1,2,3,6,7,8-HxCDF — 50 — OCDF — 100 1,2,3,7,8,9-HxCDF — 50 — OCDD — 100 2,3,4,6,7,8-HxCDF — 50 This method is also described in SW846 Method 8290 (USEPA SW846.
Data Uses/Application	The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.

Fact Sheet No.	2.1.2-17 (contd.)	
Advantages	Method 1613 is able to meet detection limits required for human health and ecological risk assessments.	
Limitations	The GC/MS portions of this method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons.	
Reference	USEPA. 1994c. Method 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, EPA 821-B-94-005. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-18
Method Title	Toxic Polychlorinated Biphenyls by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry, EPA Method 1668
Purpose	This method is for determination of the toxic Polychlorinated Biphenyls (PCBs) in water.
Method Summary	This method is performance-based. Stable isotopically labeled analogs of the toxic PCBs are spiked into a 1-L sample, and the sample is vacuum filtered through a glass-fiber filter on top of a solid-phase extraction (SPE) disk. Sample components on the filter and disk are eluded with methylene chloride and the eluant is concentrated for cleanup. After extraction, samples are cleaned up using back-extraction with sulfuric acid and/or base, and gel permeation, silica gel, Florisil and activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of specific isomers or congeners. After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, internal standards are added to each extract, and an aliquot of the extract is injected into the gas chromatography. The analytes are separated by the GC and detected by a high-resolution (≥10,000) mass spectrometer. The Method Detection Limit (MDL) for PCB #126 has been determined as 40 pg/L in water using this method. **IUPAC** — **EMDL (pg/L)** — **EML (pg/L)** 77 — 5 — 20 123, 126 — 40 — 100 118/167/156/157/169/180/170/189 — 60 — 200 114 — 600 — 2000 105 — 400 — 1000 EMD: = Estimated Method Detection Limit; EML = Estimated Minimum Level
Data Uses/Application	The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.
Advantages	Method 1668 provides data for most, but not all, of the “dioxin-like” PCBs, including those with the highest TEFs, as determined by the World Health Organization. This method provides detection limits frequently required in risk assessments.
Limitations	The GC/MS portions of this method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons. Method 1668 does not provide data for all of the “dioxin-like” PCBs, as does Method 1668A.
Reference	USEPA. 1997e. Method 1668: Toxic Polychlorinated Biphenyls by Isotope Dilution HRGC/HRMS, EPA-821-R-97-001. Office of Water, U.S. Environmental Protection Agency, Washington, DC.
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html — Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-19	
Method Title	Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, EPA Method 1668 Revision A	
Purpose	This method is for congener-specific determination of more than 150 chlorinated biphenyl (CB) congeners in water.	
Method Summary	This method is performance-based. Stable isotopically labeled analogs of the 12 PCBs designated as toxic by WHO and labeled congeners at each level of chlorination are spiked into a 1-L sample. The sample is extracted using solid-phase extraction, separatory funnel extraction, or continuous liquid/liquid extraction. After extraction, a labeled cleanup standard is spiked into the extract which is then cleaned up using back-extraction with sulfuric acid and/or base, and gel permeation, silica gel, or Florisil chromatography. Activated carbon and high-performance liquid chromatography (HPLC) can be used for further isolation of specific congener groups. After cleanup, the extract is concentrated to 20 µL. Immediately prior to injection, labeled injection internal standards are added to each extract and an aliquot of the extract is injected into the gas chromatography (GC). The analytes are separated by the GC and detected by a high-resolution (≥10,000) mass spectrometer. The estimated method detection limit (EMDL) for congener 126 in water is 5 pg/L with no interferences present. Without interferences, EMDLs and EMLs are, respectively, 5 and 10 pg/L for aqueous samples, and EMLs for extracts are 0.5 pg/uL. EMD: = Estimated Method Detection Limit; EML = Estimated Minimum Level	
Data Uses/Application	This Method is for use in data gathering and monitoring associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.	
Advantages	Method 1668A provides congener data that can be used for source identification. Listed PCBs include the 12 World Health Organization “dioxin-like” PCBs. The HRMS method provides lower EMDLs compared to ECD or low resolution MS analyses and provides unequivocal congener identification.	
Limitations	The GC/MS portions of this method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons. Solvents, reagents, glassware, and other sample processing hardware may yield artifacts, elevated baselines, and/or lock mass suppression causing misinterpretation of chromatograms.	
Reference	USEPA 1999c. Method 1668, Revision A: Chlorinated Biphenyl Cogeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, EPA-821-R-00-002. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	Rev A not available online	Last Accessed:

Fact Sheet No.	2.1.2-20	
Method Title	ESS Method 220.3: Ammonia Nitrogen and Nitrate+Nitrite Nitrogen, Automated Flow Injection Analysis Method, LMMB 061	
Purpose	This method is for the simultaneous determination of ammonia and nitrate/nitrite in surface, drinking, and ground waters, and domestic and industrial wastes.	
Method Summary	Water samples are collected and preserved with sulfuric acid. Samples are analyzed directly using an automated flow injection analyzer. For ammonia, alkaline phenol and sodium hypochlorite react with ammonia to form a blue compound that is proportional to the ammonia concentration. Ammonia is measured colorimetrically at 630 nm. For nitrate+nitrite-N, nitrate is quantitatively reduced to nitrite using a copperized cadmium column. The sample solution (total nitrite) then reacts with sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride to form a magenta solution. Total nitrite is measured colorimetrically at 520 nm. Nitrite alone can be determined by removing the cadmium column. Nitrate is quantified by subtracting the measured nitrite concentration from the measured total nitrite concentration. Samples with a concentration of 0.02-10.0 mg NH_3-N/L and 0.02-35.0 mg NO_3+NO_2-N/L can be analyzed with this method. These ranges can be extended through the use of a digital diluter. ASTM Method D1426 and Standard Method 4500-NH_3C also describe the colorimetric determination of ammonia (ASTM, 2001a; APHA, 1999). ASTM Method D 3867 and Standard Methods 4500-NO_2^-.B and 4500-NO_3^-.F describe the analysis of nitrite and nitrate.	
Data Uses/Application	The spatial and temporal variations in nutrients concentrations are often critical parameters for understanding aquatic productivity and conditions of estuarine habitat.	
Advantages	EDTA used in this procedures inhibits precipitation of residual calcium and magnesium ions. The detection range of this method includes most concentrations found in the environment.	
Limitations	Since a straight line calibration curve is not obtained, a greater number of standards is needed.	
Reference	USEPA. 1997c. Method LMMB 061: ESS Method 220.3: Ammonia Nitrogen and Nitrate+Nitrite Nitrogen, Automated Flow Injection Analysis Method, Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chemistry, and Biomonitoring Sample Analysis Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/methd220.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-21	
Method Title	ESS Method 230.1: Total Phosphorus and Total Kjeldahl Nitrogen, Semi-Automated Method, LMMB 062	
Purpose	This method is for the determination of total Kjeldahl nitrogen and total phosphorus in drinking, surface and was te waters.	
Method Sum m ary	W ater samples are collected and preserved in the field using sulfuric acid. In this method, organic nitrogen and phosphorus compounds are digested using a sulfuric acid solution containing potassium sulfate. Mercuric sulfate is used as a catalyst in the digestion. H_2SO_4 + organic nitrogen Hg $(NH_4)_2SO_4$ K_2SO_4 H_2SO_4 + organic phosphorus Hg K_3PO_4 K_2SO_4 Tubes of sam ple aliquots and acid are placed in a block digestor, where they are heated at 200°C for about 1 hour and then at 380°C for 75 minutes. The digestate is analyzed spectrophotom etrically as am m onia and phosphate using an Auto Analyzer. Total phosphorus and total Kjeldahl nitrogen concentrations are obtained directly from the plotter. Method LMMB 058 describes the general operating and maintenance procedures for using the Auto Analyzer. The Auto Analyzer is comprised of a sam pler, proportioning pump, manifold, colorimeter, and printer/plotter. The flow of reag ents and samples are proportioned by the pump, and air bubbles introduced into the tubing help to sep arate samples, mix reagents, and cleanse tubing. The operating range for this method is 0.1 to 10.0 mg N/L and 0.02 to 2.00 mg P/L. Standard Method 4500-N_{org}B and ASTM Method D3590 describe the determination of Kjeldahl nitrogen (APHA, 1999; ASTM, 2001a).	
Data Uses/Application	This method details the conversion of nitrogen compounds such as amino acids, proteins and peptides to am m onia and can be used to evaluate drinking, surface and was te waters.	
Advantages	Many water quality assessm ents require the measurem ent of total kjeldahl nitrogen.	
Limitations	The digestion process may not convert all compounds (amines, nitro compounds, hydrazones, oximes, semicarbazones, and some tertiary amines) to amm onia.	
Reference	USEPA. 1997c. Method LMMB 062: ESS Method 230.1: Total Phosphorus and Total Kjeldahl Nitrogen, Semi-Automated Method, Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chem istry, and Biomonitoring Sam ple Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
W ebs ite	http://www.epa.gov/grtlakes/lmm b/methods/ methd230.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-22	
Method Title	ESS Method 310.2: Phosphorus, Total, Low Level (Persulfate Digestion), LMMB 064	
Purpose	This method is for the determination of total phosphorus in surface waters.	
Method Summary	W ater samples are collected and preserved in the field with sulfuric acid. They are stored at 4°C until analysis. To determine dissolved phosphorus, samples are filtered through a 0.45µm filter before digestion. The samples are digested with ammonium persulfate and sulfuric acid in an autoclave for 30 minutes at 121°C. All phosphorus is converted to orthophosphate. After the digestion, any particulate matter is allowed to settle overnight. Orthophosphate is then analyzed spectrophotometrically using an Auto Analyzer. The phosphorus concentration is obtained directly from the plotter. Method LMMB 058 describes the general operating and maintenance procedures for using the Auto Analyzer. The Auto Analyzer is comprised of a sampler, proportioning pump, manifold, colorimeter, and printer/plotter. The flow of reagents and samples are proportioned by the pump, and air bubbles introduced into the tubing help to separate samples, mix reagents, and cleanse tubing. The operating range for this method is 0.002-0.200mg P/L. The persulfate digestion procedure is also described in Standard Method 4500-P.B.*5* (APHA, 1999).	
Data Uses/Application	These measurem ents are often required for water quality studies.	
Advantages	The automated analysis allows economical analyses of multiple samples.	
Limitations	This method describes only the phosphorus method.	
Reference	USEPA. 1997c. Method LMMB 064: ESS Method 310.2: Phosphorus, Total, Low Level (Persulfate Digestion), Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chemistry, and Biomonitoring Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
W ebsite	http://www.epa.gov/grtlakes/lmm b/methods/methd310.2.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-23	
Method Title	ESS Method 310.1: Ortho-Phosphorus, Dissolved Automated, Ascorbic Acid, LMMB 063	
Purpose	This method is for the determination of orthophosphate in most waters and wastewater.	
Method Summary	Water samples are collected, filtered through a 0.45 µm filter, cooled to 4°C, and analyzed as soon as possible. The samples are analyzed spectrophotometrically using an auto analyzer. In the instrument, ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of orthophosphate-phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is measured at 880 nm and is proportional to the phosphorus concentration. The phosphorus concentration is obtained directly from the plotter. Method LMMB 058 describes the general operating and maintenance procedures for using the auto analyzer. The auto analyzer is comprised of a sampler, proportioning pump, manifold, colorimeter, and printer/plotter. The flow of reagents and samples are proportioned by the pump, and air bubbles introduced into the tubing help to separate samples, mix reagents, and cleanse tubing. The operating range for this method is 0.002-0.200mg P/L. This range may be extended to 0.2-2.00 mg P/L by utilizing a dilution loop. The automated ascorbic acid reduction method is also described in Standard Method 4500-P F (APHA, 1999).	
Data Uses/Application	These measurements are useful for productivity assessments and site characterizations.	
Advantages	The auto analyzer method provides fast, reproducible nutrient results.	
Limitations	Barium, lead, and silver may interfere with the analysis by forming a precipitate.	
Reference	USEPA. 1997c. Method LMMB 063: ESS Method 310.1: Ortho-Phosphorus, Dissolved Automated, Ascorbic Acid, Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chemistry, and Biomonitoring Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/methd310.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.1.2-24
Method Title	Total Organic Carbon, Standard Method 5310
Purpose	This method is for the determination of total organic carbon in a wide variety of water samples.
Method Summary	TOC methods utilize heat and oxygen, ultraviolet irradiation, chemical oxidants, or combinations of these oxidants to convert organic carbon to carbon dioxide (CO_2). The CO_2 may be measured directly by a nondispersive infrared analyzer, reduced to methane and measured with a flame ionization detector, or CO_2 may be titrated chemically. In the Combustion-Infrared Method (5310B), the sample is homogenized and diluted as necessary, and a microportion is injected into the heated reaction chamber of a carbon analyzer, which is packed with an oxidative catalyst. The water is vaporized, and the carbon is oxidized to CO_2 and H_2O. The CO_2 from oxidation of organic and inorganic carbon is measured by means of a nondispersive infrared analyzer. This gives the measure of total carbon. TOC is obtained by the difference of total carbon and inorganic carbon (IC). IC is measured by injecting the sample into a separate reaction chamber packed with phosphoric acid-coated quartz beads. Under acidic conditions, all IC is converted to CO_2, which is measured. Under these conditions, organic carbon is not oxidized and only IC is measured. Alternatively, TOC can be measured by first acidifying the sample and purging the inorganic carbon from the sample and then measuring the remaining carbon. Other methods for measuring TOC exist, such as the Persulfate-Ultraviolet Oxidation Method (5310C) and the Wet-Oxidation Method (5310D). In both of these methods, organic carbon is oxidized to CO_2 using persulfate. ASTM Methods D6317, D2579, D4129, D4839, and D5790 and SW846 Method 9060 also describe various methods for the analysis of total organic carbon (ASTM, 2001a; USEPA SW846). Dissolved organic carbon (DOC) can be measured by first filtering the sample through a 0.45-µm-pore-diam filter. Particulate organic carbon (POC) is the fraction of TOC retained by this filter and is analyzed using a CHN elemental analyzer. The minimum detectable concentrations are 1 mg carbon/L, 0.05 mg organic carbon/L, and 0.10 mg organic carbon/L can be measured with methods 5310B, 5310C, and 5310D, respectively. Method LMMB 067 (USEPA 1997c) has an MDL of 5µg for POC.
Data Uses/Application	Site characterization. TOC is also used in the assessment of trace metal and organic contaminant data.
Advantages	One of several standard methods for TOC analysis.
Limitations	Acidification, purging, and sample blending may result in the loss of volatile organic substances. Large organic particles may fail to enter the needle used for sample injection or may oxidize slowly.

Fact Sheet No.	2.1.2-24 (contd.)	
Reference	APHA. 1999. Standard Methods for the Examination of Water and Wastewater, 20th Edition.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.1.2-25	
Method Title	Standard Operating Procedure for the Analysis of Dissolved-Phase Organic Carbon in Great Lakes Waters, LMMB 096	
Purpose	To measure dissolved organic carbon (DOC) in the filtrates of water samples.	
Method Summary	Samples are filtered immediately after collection, and stored at 4°C until analysis on the ship. Samples that are sent to the lab for analysis are frozen after arrival. Inorganic carbon is removed from the filtrate by the addition of sulfuric acid and purging of the sample with organic-free air. The samples are analyzed by conversion of organic carbon to CO_2 by an ultraviolet (UV) digester. The resulting CO_2 is detected by a non-dispersive infrared (IR) analyzer. The concentration of dissolved organic carbon is calculated using the peak height method. Alternatively, Method LMMB 066 measures DOC by high temperature (680°C) catalytic oxidation (USEPA 1997c). Inorganic carbon is first removed by acidification and purging. The CO_2 resulting from organic carbon is detected by a nondispersive infrared (IR) analyzer. This method is applicable to organic carbon concentrations from 0.2 to 50 mg/L and inorganic carbon concentrations less than 1000 mg/L. Standard Method 5310 also describes the analysis of dissolved organic carbon (APHA, 1999).	
Data Uses/Application	Dissolved and particulate metals and organic contaminant data are frequently compared to DOC.	
Advantages	Both methods provide a rapid, reproducible analytical method for DOC analysis.	
Limitations	Results for volatile organic compounds using this method may be low.	
Reference	USEPA. 1997b. Method LMMB 096: Standard Operating Procedure for the Analysis of Dissolved-Phase Organic Carbon in Great Lakes Waters, Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/docanal2.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.2-26	
Method Title	Standard Operating Procedure for the Analysis of Particulate-Phase Organic Carbon in Great Lakes Waters, LMMB 097	
Purpose	To measure particulate organic carbon (POC) in the filtrates of water samples.	
Method Summary	Samples are filtered immediately after collection through glass fiber filters. Four 12mm discs are cut from each filter and allowed to dry. The disks are folded and placed into individual tin sample containers. The disks are analyzed by catalytic combustion using an elemental analyzer (CHNS analyzer). The sample container with the disk is placed into a 1000°C furnace with a catalytic reactor tube. The sample is oxidized, and the sample gases pass through a packed chromatographic column for separation. The sample components are separated as CO_2, H_2, N_2, and H_2S. The components are detected by thermal conductivity detection (TCD). To calculate POC concentration in mg/L, the resulting mass of carbon from the four discs per sample are summed, multiplied by an area correction factor, and divided by the volume of water filtered. Alternatively, Method LMMB 067 first treats the sample filters with sulfurous acid, dries the filters at 60°C for 20-30 minutes, acidifies the filters again and dries them again for 1 hour prior to analysis (EPA 905-R-97-012c). The entire filter is analyzed using a CHN elemental analyzer. The method detection limit for this procedure is 5 ug of organic carbon remaining on a GF/F filter. The maximum amount of carbon measurable is approximately 5 mg of carbon. Standard Method 5310 also describes the analysis of particulate organic carbon (APHA, 1999).	
Data Uses/Application	Normalization of trace metal and organic contaminant data; flux measurements.	
Advantages	This method can be highly automated.	
Limitations	Results for volatile organic compounds using this method may be low.	
Reference	USEPA. 1997b. Method LMMB 097: Standard Operating Procedure for the Analysis of Particulate-Phase Organic Carbon in Great Lakes Waters, Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/pocanal2.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.2-27	
Method Title	ESS Method 140.4: Chloride - Automated Flow Injection Analysis, LMMB 060	
Purpose	This method is for the determination of chloride in drinking water, surface water, saline water, domestic and industrial wastes.	
Method Summary	Water samples are collected and analyzed within 28 days. Samples are analyzed directly using an automated flow injection analyzer. This method is based on the interaction of chlorine ions and mercuric thiocyanate. As a result, a highly colored solution is formed, which is measured colorimetrically. Samples with a concentration of 1.0-100 mg Cl/L can be analyzed directly. This range can be extended through the use of a digital diluter. Standard Method 4500-Cl^- E also describes the automated analysis of chloride (APHA, 1999). Alternative methods for measuring chlorine include titration and use of ion-selective electrode. These are described in ASTM Method D512 and Standard Methods 4500-Cl^- B, C, and D (ASTM, 2001a; APHA, 1999). The ion-selective electrode method can measure chloride concentrations up to 1000 mg Cl/L.	
Data Uses/Application	The EPA recognizes chloride in drinking water as a secondary standard.	
Advantages	This method is capable of analyzing up to 100 samples per hour.	
Limitations	Since a straight line calibration curve is not obtained, a greater number of standards is needed.	
Reference	USEPA. 1997c. Method LMMB 060: ESS Method 140.4: Chloride - Automated Flow Injection Analysis, Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chemistry, and Biomonitoring Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/methd140.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.2-28	
Method Title	ESS Method 200.5: Determination of Inorganic Anions in Water by Ion Chromatography, LMMB 059	
Purpose	This method is for the determination of chloride, nitrate-N, and sulfate in drinking water, surface water, and mixed domestic and industrial wastewater.	
Method Summary	Water samples are collected and preserved as follows: Chloride: No preservation required. Analyze within 28 days. Nitrate-N: Cool to 4°C. Analyze within 48 hours. Sulfate: Cool to 4°C. Analyze within 28 days. Samples are filtered through a 0.45 µm filter to remove particulate matter. A portion of the sample (usually 5 mL) is injected into the ion chromatography, comprised of a guard column, separator column, suppressor column, and conductivity detector . The anions are separated based on their affinity for the exchange sites of the resin in the analytical and guard column. Anions are identified base on their retention times compared to known standards. Results are reported as mg/L. Alternative methods for the determination of anions (including additional anions, such as phosphate and nitrite) by Ion Chromatography are Standard Method 4110B, ASTM D4327, and SW846 9056 (APHA, 1999; ASTM, 2001a;USEPA SW846).	
Data Uses/Application	Site characterization/site assessment.	
Advantages	The suppressor column reduces background conductivity.	
Limitations	Phosphate is not analyzed with this method. Nitrite can interfere with the detection of chloride.	
Reference	USEPA. 1997c. Method LMMB 059: ESS Method 200.5: Determination of Inorganic Anions in Water by Ion Chromatography, Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chemistry, and Biomonitoring Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/methd200.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.2-29	
Method Title	Standard Operating Procedure for Electrometric pH, LMMB 092	
Purpose	To measure pH in drinking, surface, and saline waters; domestic and industrial wastes.	
Method Summary	The working range of this method is 6.0 to 10.0 pH units. Samples are collected in clean glass or plastic containers and stored at 4°C until analysis. The pH meter is calibrated with 7.0 and 10.0 buffers. The sample is brought to 25°C before analysis. An aliquot of the sample is placed into a suitable container, which is then placed on a stirrer. The pH meter electrode is submerged into the sample and the pH reading is taken once the meter stabilizes. ASTM Method D1293, Standard Method SM 4500-H^+.B, and SW846 Method 9040B also describe the measurement of pH of water (ASTM, 2001a; APHA, 1999).	
Data Uses/Application	The pH of water is an important parameter in the solubility of trace minerals in water and the suitability of the water to sustain life.	
Advantages	Little skill and training is needed to perform this analysis.	
Limitations	Temperature affects the electrometric response and must be compensated for. Extremely acidic waters require a different calibration range (*i.e.,* 4.0 and 7.0 buffers).	
Reference	USEPA. 1997c. Methods LMMB 092: Standard Operating Procedure for Electrometric pH, Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/phydrion.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.2-30	
Method Title	Standard Operating Procedure for GLNPO Total Alkalinity Titration, LMMB 091	
Purpose	To measure alkalinity in drinking, surface, and saline waters; domestic and industrial wastes.	
Method Summary	This method is designed for waters in the range of 10 - 250 mL/L total alkalinity as $CaCO_3$. The pH meter is calibrated with 4.0 and 7.0 buffers. 100mL of sample are titrated with 0.0200 N sulfuric acid to pH 4.5. Total alkalinity is calculated as $CaCO_3$ in mg/L by multiplying the volume of titrant (in mL) by 10. Similar methods for the measurement of alkalinity are described in ASTM Methods D1067 and D3875 and Standard Method 2320B (ASTM, 2001a; APHA, 1999).	
Data Uses/Application	Alkalinity is a measure of water's natural buffering capacity, thus an important parameter measured to assess overall water quality.	
Advantages	This method can be automated or semi-automated for multiple analyses.	
Limitations	Oil, grease, and high mineral content may interfere with the alkalinity determination.	
Reference	USEPA. 1997b. Methods LMMB 091: Standard Operating Procedure for GLNPO Total Alkalinity Titration, Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/alkali.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.2-31	
Method Title	Standard Operating Procedure for GLNPO Specific Conductance: Conductivity Bridge, LMMB 094	
Purpose	To measure conductance of drinking and surface waters.	
Method Summary	Samples are collected and stored at 4°C until analysis. The specific conductance of the samples is measured using a self-contained conductivity meter. Care should be taken to assure that not air bubbles are present in the conductivity cell. The temperature is adjusted to 25°C and the conductivity read. The approximate working range of this method of 10-500 mhos/cm. ASTM Method D1225, Standard Method 2510B, and SW846 Method 9050A describe similar methods for the measurement of conductivity of water (ASTM, 2001a; APHA, 1999). The test range of Method D1225A is 10-200000 µS/cm.	
Data Uses/Application	This method is applicable to the quantitative measurement of ionic constituents dissolved in water.	
Advantages	The procedure and equipment are simple and easy to operate.	
Limitations	Sample temperatures other than 25°C will cause incorrect results. Oil, grease, algae, or dirt may interfere with the readings.	
Reference	USEPA. 1997b. Method LMMB 094: Standard Operating Procedure for GLNPO Specific Conductance: Conductivity Bridge, Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/conducti.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.2-32	
Method Title	Standard Operating Procedure for GLNPO Turbidity: Nephelometeric Method, LMMB 090	
Purpose	To measure turbidity in drinking, surface, and saline waters.	
Method Summary	Turbidity samples are analyzed immediately or stored at 4°C. The working range of the turbidimeter is 0-20 nephelometeric turbidity units (NTU). Dilutions can be performed to measure turbidities greater than 20 NTU. The instrument is calibrated with a geometric series of calibration standards. An aliquot of the sample is warmed to 25°C and placed into the turbidimeter for measurement. The instrument measures turbidity by comparing the intensity of light scattered by the sample with the intensity of light scattered by a standard reference suspension. ASTM Method D1889 and Standard Method 2130B describe a similar method of measuring the turbidity of water (ASTM, 2001a; APHA, 1999). These methods have a working range of 1.0-40 NTU.	
Data Uses/Application	Turbidity is measured as part of overall site characterization, as an indirect measure of light penetration. Turbidity can also be monitored during dredging or other sediment excavations to measure amounts of suspended material entering the environment for site activities.	
Advantages	Turbidity measured with a nephelometer provides a much more rapid and reproducible measurement compared to the filtration/color chart method.	
Limitations	Floating debris and air bubbles in the sample may give high readings. Condensation or scratches on the sample vial and sample color may give erroneous readings.	
Reference	USEPA. 1997b. Methods LMMB 090: Standard Operating Procedure for GLNPO Turbidity: Nephelometeric Method, Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/turbid.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.2-33	
Method Title	ESS Method 340.2: Total Suspended Solids, Mass Balance (Dried at 103-105°C) Volatile Suspended Solids (Ignited at 550°C), LMMB 065	
Purpose	To measure the portion of total solid retained by a filter from drinking, surface, and saline waters; domestic and industrial wastes.	
Method Summary	Water samples are collected by submersible pump or Rosette sampler. A sample volume is selected that will yield 2 - 20,000 mg/L. total suspended solids. For open-lake oligotrophic conditions, 2-4 liters will provide enough particulate matter. For near-shore or eutrophic conditions, 200-500 mL may be sufficient. A well-mixed sample is filtered through a preweighed standard glass-fiber filter, and the residue retained on the filter is dried at 103 to 105 °C for at least one hour. The increase in weight of the filter represents the total suspended solids. After determining TSS, the filters may be placed in a muffle furnace and ignited at 550°C for 30 minutes to determine volatile suspended solids (VSS). Following Method LMMB 098, water samples can be filtered in the field and then frozen at -10°C until final weighing in the laboratory. Standard Method 2540D, ASTM Method D5907, and Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies describe similar methods for measuring total suspended solids (APHA, 1999; ASTM, 2001a; USEPA 1992b, respectively).	
Data Uses/Application	TSS is an ancillary parameter to the determination of hydrophobic organic contaminants (HOCs).	
Advantages	Glass fiber filters can be ignited without damage, allowing TSS and VSS to be performed on the same set of filters.	
Limitations	Excessive residue on the filter may form a water-entrapping crust. Sample size should be limited to yield no more than 200 mg residue. Glass fiber filters are not appropriate for measurement of TSS in estuarine waters.	
Reference	USEPA. 1997c. Method LMMB 065: ESS Method 340.2: Total Suspended Solids, Mass Balance (Dried at 103-105°C) Volatile Suspended Solids (Ignited at 550°C). Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chemistry, and Biomonitoring Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/methd340.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.2-34	
Method Title	Total Hardness Titration, LMMB 095	
Purpose	To measure the total concentration of the calcium and magnesium ions expressed as calcium carbonate.	
Method Summary	A water sample is collected at mid depth during unstratified conditions, or on the mid-epilimnion and mid hypo-limnion when stratification is present. A 100 mL water sample is buffered to pH 10.1, and an indicator (such as Chrome Black T3) is then added to the buffered sample. The indicator turns red in the presence of Ca and Mg ions. The sample is titrated with 0.01M EDTA, which complexes with Mg and Ca cations, removing them from association with the indicator. When all the Mg and Ca are complexed with EDTA, the indicator will turn blue. The volume of titrant is recorded. Total Hardness is calculated as 10 X mL of titrant and reported as mg/L as $CaCO_3$. Standard Method 2340C and ASTM Method D1126 also describe the titration analysis of hardness (APHA, 1999; ASTM, 2001a). An alternative method for the analysis of hardness is to determine the amount of calcium and magnesium ions separately and then sum them to calculate total hardness. Standard Method 2340B and ASTM Method D1126 both describe this alternative method (APHA, 1999; ASTM, 2001a). When determined as separate ions, hardness is calculated as: 2.497[Ca, mg/L] + 4.118 [Mg, mg/L]	
Data Uses/Application	Hardness is measured in aquatic systems because it is known to mitigate metals toxicity in fish.	
Advantages	The reagents and chemicals required for this analysis can be obtained as prepackage test kits. The titration method affords a means of rapid analysis, where as the calculation method is more accurate.	
Limitations	This test method is not suitable for highly colored waters, which obscure the color change of the indicator. A limit of 5 minutes is set for the duration of the titration to minimize the tendency toward CaCO3 precipitation.	
Reference	USEPA. 1997c. Method LMMB 095: Total Hardness Titration. Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chemistry, and Biomonitoring Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/grtlakes/lmmb/methods/titratio.pdf	Last Accessed: 1/23/2003

2.1.3 Biological Analysis Methods

Section 2.1.3 provides a compendium of water-related biological analyses. As mentioned previously, once contaminants enter into an aqueous system, the contaminant's chemical nature and the biological, chemical, or physical characteristics of the receiving water body will determine whether it remains in the water column, becomes buried in sediment, or is ingested by organisms. The negative impacts of contaminant exposure can be examined in laboratory toxicity tests, which use site-specific effluents, leachates or elutriates prepared from sediments collected from the site. All liquid-phase toxicity tests are performed to ultimately determine the lowest observable effect concentration, the no observable effect concentration and other related parameters. These results are then compared with chemistry data to identify and compare toxicity effects data with contaminant exposure data to determine risk to ecological or human receptors. While elutriate toxicity testing is performed to evaluated sediment toxicity, they are included in this section because the same aqueous phase methods are also used to evaluate water and wastewater.

The advantages and limitations associated with each test are provided in their respective fact sheets. However, there are general limitations in interferences associated with all liquid-phase toxicity tests. These potential interferences are identified by some of the source documents (USEPA, 1994d; Weber, 1991). They are listed below:

- Toxicity tests do not reflect temporal fluctuations in effluent toxicity;
- Non-target chemicals can cause adverse effects to the organisms giving false results;
- dissolved oxygen depletion due to biological and chemical oxygen demand and metabolic wastes can be a problem;
- The toxicant may be lost through volatilization and adsorption to the exposure chamber; and,
- The effect of the toxicant is organism dependant.

Liquid-phase toxicity tests vary considerably in test length, endpoints, and test species. Table 2.1.3-1 summarizes those toxicity tests described in the fact sheets, however it highlights the differences between specific organisms to aid the Superfund manager with selecting the most appropriate test for his/her site.

The toxicity test fact sheets provide methods described in USEPA guidance documents, Dredging Manuals and ASTM reports. Specifically, the following sources provided methods information for section 2.1.3:

- The USEPA's Environmental Response Team SOP's (USEPA , 1994b); ERT SOPs are available online at: http://www.ert.org www.epa.gov/waterscience/WET/disk1/
- The USEPA's Methods document for Effluents and Receiving waters (Weber, 1991);
- The USEPA's guidance document for contaminated sediment assessment in the Great Lakes (USEPA, 1993a);
- The Inland Lakes Testing Manual (USEPA and USACE, 1998);
- The USEPA's Environmental Research Laboratory-Narragansett (USEPA and the Naval Construction Battalion Center, 1992)

Table 2.1.3-1. A Summary of Test Types and Toxicological Endpoints for Liquid-Phase Toxicity [1, 2]

Test Type/Fact Sheet Number	Test Organism	Scientific Name	Endpoints	Test Specifics	Comments
Acute Freshwater 2.1.3-1	Crustacean	*Daphnia magna*	Survival	Static, static-renewal, 24, 48 or 96 hours	Commonly used for bioassays; optimum pH 6.8-8.5
Acute Freshwater 2.1.3-1	Crustacean	*Daphnia Pulex*	Survival	Static, static-renewal, 24, 48 or 96 hours	Commonly used for bioassays; optimum pH 6.8-8.5
Acute Freshwater 2.1.3-2	Fish	*Pimephales Promelas*	Survival	Static, static-renewal, 24, 48 or 96 hours	Other commonly used freshwater fish in bioassays include the *Oncorhynchus mykiss*
Chronic Freshwater 2.1.3-3	Algae	*Selanastrum capricornutum*	Growth, biostimulatory effects (cell density	Static, 96 hours	Originally designed as a eutrophication test, now used in the Superfund program for effluents.
Chronic Freshwater 2.1.3-4	Crustacean	*Ceriodaphnia dubia*	Survival, reproduction	Static renewal, 7 days	Commonly used for bioassays
Chronic Freshwater 2.1.3-5	Crustacean	*Daphnia magna*	Reproduction, growth	Static-renewal, 10 days	Commonly used for bioassays; optimum pH 6.8-8.5
Chronic Freshwater 2.1.3-5	Crustacean	*Daphnia Pulex*	Reproduction, growth	Static-renewal, 10 days	Commonly used for bioassays; optimum pH 6.8-8.5
Chronic Freshwater 2.1.3-6	Fish	*Pimephales promelas*	Larval Survival and growth	Static-renewal, 7 days	Other commonly used freshwater fish in bioassays include the *Oncorhynchus mykiss*
Chronic Freshwater 2.1.3-17	Fish	*Pimephales promelas, Ictalurus punctatus, Coregonus artedii, Oryzias latipes, Catastomus commersoni, Esox lucius, Danio danio*	Survival, growth	Single exposure, 32 days (100 days for *Coregonus artedii*)	One of the few, published early life stage methods
Acute Marine 2.1.3-7	Macroalgae	*Champia parvula*	Sexual reproduction	Static, 48 hours	Used for whole effluent toxicity testing in the NPDES Program
Acute Marine 2.1.3-8	Crustacean	*Mysidoposis bahia*	1-25 ppt	Static-renewal, 96 hours	Other commonly used estuarine/marine mysid shrimp in bioassays include the *Holmesimysis costata* and the *Neomysis americana*
Acute Marine 2.1.3-9	Fish	*Cyprinodon variegatus*	Survival	Static-renewal, 24, 48 or 96 hours	Commonly used in bioassays
Acute Marine 2.1.3-9	Fish	*Menidia beryllina*	Survival	Static-renewal, 24, 48 or 96 hours	Commonly used in bioassays

Table 2.1.3-1. (contd.)

Test Type	Test Organism	Scientific Name	Endpoints	Test Specifics	Comments
Chronic Marine 2.1.3-10	Crustacean	*Mysidopsis bahia*	Survival, growth, fecundity	Static-renewal, 7 days	Other commonly used estuarine/marine mysid shrimp in bioassays include the *Holmesimysis costata and Neomysis americana*
Chronic Marine 2.1.3-11	Echinoderm	*Arbacia punctulata*	Toxicity to eggs and sperm/ % fertilization	One hour	Chronic test but test time is quite short.
Chronic Marine 2.1.3-12	Fish	*Cyprinodon variegatus*	Larval survival and growth	static-renewal, 7 days	Commonly used in bioassays
Chronic Marine 2.1.3-12	Fish	*Menidia beryllina*	Larval survival and growth	static-renewal, 7 days	Commonly used for bioassays

[1] Three species are generally recommended for water column bioassays. They should represent different phyla where possible.
[2] Specific characteristics to consider when selecting water-column test species include availability year-round, tolerance to handling and laboratory conditions, consistent and reproducible responses to toxicants, phylogenic similarities to species characteristic of those inhabiting water column at impacted area, availability of standardized test protocols, ability to test as juveniles or larvae to increase sensitivity, appropriate sensitivity.

Fact Sheet No.	2.1.3-1
Method Title	Acute Freshwater Crustacean Bioassay: 48 Hours, ERT SOP 2024
Purpose	This static toxicity test measures the survival of the freshwater crustaceans, *Daphnia Magna* and *Daphnia Pulex*, after exposure to leachates, effluents, or liquid phases of sediments (*i.e.,* elutriates or pore water) for 48 hours in the laboratory.
Method Summary	Larval daphnids, *Daphnia Magna* and *Daphnia Pulex*, are exposed to various concentrations of liquid-phase test media for 48 hours in 100-mL containers (or 250 mL, USEPA 1993a). The media concentrations levels intend to span a range of those causing zero mortality to those causing complete mortality. If the test medium is a liquid, dilution may be made directly for the required test concentrations. If the test medium is a sediment, preliminary filtration and dilutions are required to produce a liquid phase that will then be diluted to attain the above test concentrations. Once all the exposure chambers are set up with their designated test concentrations, the test organisms are added after being acclimated to the dilution water in separate chambers. The experiment officially begins when half of the organisms are in the exposure chambers. Test temperature is 20.0 +/- 2 $^{\circ}$C for the daphnids. The test is static; water will not be renewed throughout the duration of the test. The endpoints are survival at 1-hour, 24-hours, and 48-hours. Organisms are not fed during the test. At the termination of the test, mortality and water quality parameters are recorded. Range-finding tests may be performed prior to these analyses to determine the appropriate test media dilutions. Reference toxicant tests will also be performed simultaneous to these tests. The acute toxicity tests described for use in the NPDES Permits Program indicate that this test may be static or static-renewal and it is run for 24, 48 or 96 hours (Weber, 1991). A similar method for acute toxicity using *Daphnia* spp. is described in the Environment Canada Method EPS 1/RM/11.
Data Uses/Application	The results are used to determine the lethal concentration for 50% of the test species (LC_{50}). The Lowest Observable Effects Concentration (LOEC) and the No Observable Effects Concentration (NOEC) are also recorded.
Advantages	Both of these species of daphnids are considered sensitive benchmark species (USEPA and USACE 1998). Benchmark species comprise a substantial data base, represent the sensitive range of a variety of ecosystems and provide comparative data on the relative sensitivity of local test species. Liquid-phase toxicity testing is often simpler to run than bulk sediment testing; excellent correspondence between bulk sediment contaminant concentrations and pore water toxicity has been observed.

Fact Sheet No.	2.1.3-1 (contd.)	
Limitations	Daphnia are freshwater crustaceans, therefore they cannot be used in estuarine and marine settings. The optimum pH range for Daphnids is 6.8 to 8.5; therefore, the pH of the dilution water or the concentrations may have to be adjusted prior to the start of the test.	
Reference	USEPA. 1994b. 48-Hour Acute Toxicity Test Using Daphnia Magna and Daphnia Pulex, SOP #2024. Compendium of ERT Standard Operating Protocols. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison NJ.	
Website	http://www.ert.org/products/2024.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.3-2	
Method Title	Acute Freshwater Fish Bioassay, ERT SOP 2022	
Purpose	These methods describe a static-renewal toxicity test using the freshwater fish, *Pimephales promelas*. These tests are effective when testing the acute toxicity of effluents, leachates and liquid phases of sediments for 96 hours in the laboratory.	
Method Summary	The larval *Pimephales promelas* (fathead minnow) is exposed to various concentrations of test media over a 96-hour period in 1-L test containers. The medium concentration level is planned to span a range of those causing zero mortality to those causing complete mortality. If the test medium is a liquid, dilution may be made directly for the required test concentrations. If the test medium is a sediment, preliminary filtration and dilutions are required to produce a liquid phase that will then be diluted to attain the above test concentrations. The test temperature is 25 +/- 2 °C. Fish will be fed during the acclimation period and during the toxicity test. Test solutions will be replaced daily in exposure chambers. Record survival at one hour and then daily thereafter. After the 96 hours has past, final mortality and water quality measurements are recorded. Range-finding tests may be performed prior to these analyses to determine the appropriate test media dilutions. Reference toxicant tests will also be performed simultaneous to these tests. The acute freshwater fish bioassay used in the NPDES permits program indicates that this test may be static or static-renewal and run for 24, 48 or 96 hours (Weber. 1991). The rainbow trout, *Oncorhynchus mykiss*, is another benchmark freshwater fish specie used for water column toxicity tests (USEPA and USACE. 1998).	
Data Uses/Application	This test may be conducted on effluents, leachates, or liquid phase of sediments. The results will be used to determine the lethal concentration of test media that causes 50% mortality (LC_{50}). The Lowest Observable Effect Concentration (LOEC) and the No Observable Effects Concentration (NOEC) is also recorded.	
Advantages	*Pimephales promelas* are easily reared in the laboratory and they are important forage fish in the food chain. *Pimephales promelas* are considered benchmark species indicating that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species (USEPA and USACE, 1998).	
Limitations	Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	USEPA. 1994b. 96-Hour Acute Toxicity Test Using Larval Fathead Minnows (*Pimephales proomelas*) SOP #2022. Environmental Response Team. Compendium of ERT Standard Operating Protocols. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison NJ.	
Website	http://www.ert.org/products/2022.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.3-3
Method Title	Chronic Freshwater Algae Test, ERT SOP 2027
Purpose	This test measures the biostimulatory capabilities of leachates, effluents, or liquid phases of sediments (*i.e.,* elutriates or pore water) on *Selenastrum capricornutum* for 96-hour exposure of in the laboratory.
Method Summary	The freshwater algae, *Selenastrum capricornutum* is exposed to various concentrations of test media over a 96-hour period in 100-mL test containers. The media concentration levels intend to span a range of those causing zero mortality to those causing complete mortality. If the test medium is a liquid, dilution may be made directly for the required test concentrations. If the test medium is a sediment, preliminary filtration and dilutions are required to produce a liquid phase that will then be diluted to attain the above test concentrations. Test temperature is 25 +/- 2 ° C. The endpoint is growth. There is no water renewal throughout the test. Growth is measured at the end of the test by cell counts, chlorophyll content or turbidity (light absorbance), or biomass. Cell counts are determined using an automatic particle counter or manually under a microscope. Chlorophyll content may be measured using in-vivo or in-vitro fluorescence or in-vitro spectrophotometry. Turbidity is measured by spectrophotometry at 750 nm. Biomass is measured by multiplying the cell count by the mean cell volume or by direct gravimetric dry weight analysis. This test can also be conducted for 24-hours using 25-mL glass borosilicate tubes with the following dilutions: 0%, 10%, 25%, 50% and 94% elutriate. This test protocol is also described in EPA guidance documents for effluents and receiving waters and assessing contaminated sediments in the Great Lakes (Weber, 1993; USEPA, 1993a). Environment Canada methods EPS 1/RM/25 and EPS 1/RM/37 describe similar tests using *Selenastrum capricornutum* and *Lemna minor*.
Data Uses/Application	This method was originally designed to test for eutrophication, however it has been recommend for use in testing the toxicity of complex effluents and has been widely used to test single chemicals. The results of this test will be used to determine the No Observable Effect Concentration (NOEC), Lowest Observable Effect Concentration (LOEC) and the Chronic Value (CHV). These results will determine the long term effects of those sediment samples on the surrounding biotic community.
Advantages	There is currently no method for exposing algae directly to whole sediments, thus an elutriate most closely simulates the most likely exposure conditions for natural algal populations.

Fact Sheet No.	2.1.3-3 (contd.)	
Limitations	The concentration of natural nutrients in the test media may affect the results. This is not a benchmark test species used for toxicity testing (USEPA and USACE, 1998). Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	USEPA. 1994b. 96-Hour Static Toxicity Test Using *Selenastrum capricornutum*, SOP #2027, Environmental Response Team. Compendium of ERT Standard Operating Protocols. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison NJ.	
Website	http://www.ert.org/products/2027.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.3-4
Method Title	Chronic Freshwater Crustacean Bioassay (7 Day), ERT SOP 2025
Purpose	These methods describe a 7-day, static-renewal toxicity test used to measure chronic effects from liquid-phase test media on the freshwater crustacean, *Ceriodaphnia dubia*.
Method Summary	The freshwater water flea*, Ceriodaphnia dubia,* are exposed to various concentrations of test media over a 7-day period in 30mL test chambers. The media concentration levels intend to span a range of those causing zero mortality to those causing complete mortality. If the test medium is a liquid, dilution may be made directly for the required test concentrations. If the test medium is a sediment, preliminary filtration and dilutions are required to produce a liquid phase that will then be diluted to attain the desired test concentrations. Test temperature is 25 +/- 2° C. New test media concentrations are prepared daily. The organisms are physically transferred to newly prepared exposure chambers. Organisms are fed daily. Survival is recorded over a 7-day period as well as the number of broods and the brood size. The number of males surviving are counted at test termination. Range-finding tests may be performed prior to these analyses to determine the appropriate test media dilutions. Reference toxicant tests will also be performed simultaneous to these tests. Similar freshwater crustacean species such as the *Daphnia magna* and the *Daphnia pulex* may also be used for similar assays. The EPA guidance documents for toxicity testing of effluents and receiving waters also describes this method (Weber. 1991), as does Environment Canada's method EPS 1/RM/21.
Data Uses/Application	The data from these tests will be used to determine the Lowest Observable Effect Concentration (LOEC), the No Observable Effect Concentration (NOEC), the EC_{50} and the chronic value of the test media.
Advantages	*Ceriodaphnia dubia* are commonly used test species for freshwater toxicity testing, therefore resulting data will be comparable to a large number of previous studies. These organisms are benchmark species indicating that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species(USEPA and USACE, 1998).
Limitations	*Ceriodaphnia dubia* are freshwater species; they can only be used when salinity is <1 ppt. Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.

Fact Sheet No.	2.1.3-4 (contd.)	
Reference	USEPA. 2002. 7-Day Static Renewal Toxicity Test Using Ceriodaphnia dubia. Rev 1. SOP #2025, Environmental Response Team. Compendium of ERT Standard Operating Protocols. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison NJ.	
Website	http://www.ert.org/products/2025.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.3-5	
Method Title	Chronic Freshwater Crustaceans Bioassay (10 days), ERT SOP 2028	
Purpose	These methods describe a 10-day, static renewal toxicity test used to measure chronic effects from contaminated test media on the freshwater daphnids, *Daphnia magna* or *Daphnia pulex*	
Method Summary	Larval *Dapnia magna* or *Daphnia pulex* are placed in individual 100-mL containers and exposed to different concentrations of liquid-phase test media over a 10-day period. If the test medium is a liquid, dilution may be made directly for the required test concentrations. If the test medium is a sediment, preliminary filtration and dilutions are required to produce a liquid phase that will then be diluted to attain the desired test concentrations. Test temperature is 25 degrees +/- 2 °C. Test media concentrations are renewed every other day for the duration of the test; organisms are physically transferred into new exposure chambers. Organisms are fed every other day. The endpoints of the test are mortality, reproduction and growth. *Ceriodaphnia dubia* is also recommended as a freshwater daphnid that may be used for similar assays (USEPA and USACE, 1998).	
Data Uses/Application	This test is applicable to leachates, effluents, and liquid phases of sediments. The data from these tests will be used to determine the Lowest Observable Effect Concentration (LOEC), the No Observable Effect Concentration (NOEC), the EC50 and the chronic value of the test media.	
Advantages	All three of these species of daphnids are considered sensitive benchmark species (USEPA and USACE 1998). Benchmark species comprise a substantial data base, represent the sensitive range of a variety of ecosystems and provide comparative data on the relative sensitivity of local test species.	
Limitations	Daphnia are freshwater crustaceans, therefore they cannot be used in estuarine and marine settings. The optimum pH range for daphnids is 6.8 to 8.5; therefore, the pH of the dilution water or the concentrations may have to be adjusted prior to the start of the test. Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	USEPA.1994b. 10-day Chronic Toxicity Test using *Daphnia Magna* or *Daphnia Pulex*. SOP #2028. Environmental Response Team. Compendium of ERT Standard Operating Protocols. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison NJ.	
Website	http://www.ert.org/products/2028.pdf	Last Accessed: 1/23/2003

Fact Sheet No.	2.1.3-6	
Method Title	Chronic Freshwater Fish Bioassay, ERT SOP 2026	
Purpose	These methods describes a 7-day, static-renewal toxicity test using the larval freshwater fish, *Pimephales promelas*.	
Method Summary	The *Pimephales promelas* are exposed to various concentrations of liquid-phase test media over a 7-day period in 500 mL - 1L test containers. The media concentration levels intend to span a range of those causing zero mortality to those causing complete mortality. If the test medium is a liquid, dilution may be made directly for the required test concentrations. If the test medium is a sediment, preliminary filtration and dilutions are required to produce a liquid phase that will then be diluted to attain the desired test concentrations. Test temperature is 25 +/- 2 °C. The fish are fed daily at 4 hour intervals. New dilutions of test media are prepared daily. The old solution is drawn out with a siphon and the newly prepared solutions are added to each chamber. The endpoints are survival and growth. Range-finding tests may be performed prior to these analyses to determine the appropriate test media dilutions. Reference toxicant tests will also be performed simultaneous to these tests. The rainbow trout, *Oncorhynchus mykiss*, is another benchmark freshwater fish specie used for water column toxicity tests. A method for testing acute toxicity using rainbow trout can be found in Environment Canada's method EPS 1/RM/9. EPA guidance for toxicity testing of effluents and receiving waters also describes this method (Weber, 1991, USEPA, 1990b), as does Environment Canada's method EPS 1/RM/22.	
Data Uses/Application	The results of this test will be used to determine the No Observable Adverse Effect Concentration (NOAEC), Lowest Observable Adverse Effect Concentration (LOAEC) and the Chronic Value (CHV). These results will determine the long term effects of those sediment samples on the surrounding biotic community.	
Advantages	*Pimephales promelas* are easily reared in the laboratory and they are important forage fish in the food chain. *Pimephales promelas* are considered benchmark species indicating that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species (USEPA and USACE, 1998).	
Reference	USEPA. 2002. 7-Day Static Toxicity Test Using Larval *Pimephales promelas*. Rev 1. SOP #2026. Environmental Response Team. Compendium of ERT Standard Operating Protocols. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison NJ.	
Website	http://www.ert.org/products/2026.pdf	Last Accessed: 2/12/03

Fact Sheet No.	2.1.3-7	
Method Title	Chronic Marine Macroalgae, *Champia parvula*, Sexual Reproduction Test, NHEERL-AED SOP 1.03.001	
Purpose	This toxicity test measures the effects of toxic substances in effluents and receiving waters on the sexual reproduction of the marine macroalga, *Champia parvula*, during a forty-eight hour exposure.	
Method Summary	Macroalga are exposed to different concentrations of test concentrations of effluent test medium over a 48-hour period. The selection of the effluent test concentrations should be based on the objectivity of the test, however the maximum effluent concentration which can be tested is 50% and the dilution factors may range from 0.5 to 0.3. Three test chambers are devoted to effluent treatment and three chambers are controls. Each test chamber will be filled with 100 mL of control or treatment water and five female branches and one male branch. The water is not renewed throughout the test, however each chamber is hand-swirled twice a day to mix the water column. After 48 hours, the organisms are removed and placed in recovery bottles. Investigators then count the cystocarps under a stereomicroscope. NPDES Guidance suggest a 5-7 day test duration (Weber, 1991).	
Data Uses/Application	This is a laboratory test applicable to testing toxicity of effluents and receiving waters. The results are used to determine the sexual reproduction ability of the macroalgae exposed to different dilutions of the test medium.	
Advantages	This is one of few toxicity methods developed using marine plants.	
Limitations	The salinity of the test water must be 30 ppt +/- 2 ppt. Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296, Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.03.001.pdf	Last Accessed:

Fact Sheet No.	2.1.3-8	
Method Title	Acute Marine Crustacean Bioassay, NHEERL-AED SOP 1.03.003	
Purpose	This static toxicity test measures the survival of the marine crustacean, *Mysidopsis bahia*, after exposure to effluents and receiving waters for 96 hours in the laboratory.	
Method Summary	Mysids are exposed to various concentrations (minimum of 5) of effluent test medium over a 96-hour period in 250 mL containers. The effluent concentrations are commonly selected to approximate a geometric series (*i.e.,* a dilution factor of 0.5). At least 20 organisms of a given species are exposed to each effluent concentration. Test temperature is 20 +/- 2 °C. Organisms will be fed during the acclimation period and during the toxicity test. Record survival at one hour and then daily thereafter. After the 96 hours has past, final mortality and water quality measurements are recorded. Range-finding tests may be performed prior to these analyses to determine the appropriate test media dilutions. Reference toxicant tests will also be performed simultaneous to these tests. Other mysid shrimp species used for water column bioassays include *Neomysis americana* and the *Holmesimysis costata*.	
Data Uses/Application	This test may be conducted on effluents, leachates, or liquid phase of sediments. The results will be used to determine the lethal concentration of test media that causes 50% mortality (LC_{50}).	
Advantages	Mysid shrimp are considered sensitive benchmark species (USEPA and USACE, 1998). Benchmark species comprise a substantial data base, represent the sensitive range of a variety of ecosystems and provide comparative data on the relative sensitivity of local test species.	
Limitations	Mysid shrimp are near coastal species; they are used for testing in marine/estuarine systems with salinities between 15 and 30 ppt (ASTM Standard Method E1191; ASTM, 2001b.) Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296, Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.03.003.pdf	Last Accessed:

Fact Sheet No.	2.1.3-9	
Method Title	Acute Marine Fish Bioassay, NHEERL-AED SOP 1.03.003	
Purpose	These methods describe a 96-hour, static renewal acute effluent toxicity test using the marine fish species *Menidia beryllina* and *Cyprinodon variegatus*	
Method Summary	Marine fish species, *Menidia beryllina* and *Cyprinodon variegatus* are exposed to various concentrations (minimum of 5) of liquid-phase test media over a 96-hour period in 250 mL containers. The test media concentrations are commonly selected to approximate a geometric series (*i.e.,* a dilution factor of 0.5). At least 20 organisms of a given species are exposed to each effluent concentration. Both species are generally used in salinities greater than 25 ppt. Test temperature is 20 +/- 2°C. Organisms will be fed during the acclimation period and during the toxicity test. Test solutions must be replaced daily in the exposure chambers. Survival is recorded at one hour and then daily thereafter. After the 96 hours have passed, final mortality and water quality measurements are recorded. Range-finding tests may be performed prior to these analyses to determine the appropriate test media dilutions. Reference toxicant tests will also be performed simultaneous to these tests. A similar effluent toxicity test is also described in EPA guidance pertaining to effluents and receiving waters (Weber, 1991).	
Data Uses/Application	This test may be conducted on effluents, leachates, or liquid phase of sediments. The results will be used to determine the lethal concentration of test media that causes 50% mortality (LC_{50}).	
Advantages	Both fish species are considered benchmark species indicating that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species (USEPA and USACE, 1998).	
Limitations	Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296, Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.03.003.pdf	Last Accessed:

Fact Sheet No.	2.1.3-10	
Method Title	Chronic Estuarine Survival, Growth and Fecundity Test, NHEERL-AED SOP 1.03.005	
Purpose	These methods describe a toxicity test used to measure chronic effects from effluents and receiving waters on the estuarine mysid, *Mysidopsis bahia* during a seven-day, static-renewal exposure.	
Method Summary	The estuarine mysid shrimp, *Mysidopsis bahia,* are exposed to various concentrations of test media over a 7-day period in 200 mL glass beakers. The media concentration levels intend to span a range of those causing zero mortality to those causing complete mortality. 150 mL of the appropriate effluent dilution is added to each beaker. The test can be run with smaller volumes of water as well. (Ho, 2000). Test temperature range is 26 - 27° C. New test media concentrations are prepared daily. The test organisms are fed daily. Mortality is recorded over a 7-day period. Following the test, the live animals are examined for eggs and the sexes are determined within 12 hours of the test termination. Other mysid shrimp species used in similar analyses include *Neomysis americana* and *Holmesimysis costata*. A similar effluent toxicity test is also described in EPA guidance pertaining to effluents and receiving waters (Weber, 1991)	
Data Uses/Application	The data from these tests will be used to determine the Lowest Observable Effect Concentration (LOEC), the No Observable Effect Concentration (NOEC), the EC_{50} and the chronic value of the test medium.	
Advantages	*Mysidopsis bahia* are benchmark species indicating that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species (USEPA and USACE, 1998).	
Limitations	*Mysidopsis bahia* are near coastal species, They are used for salinities between 15 and 30 ppt (ASTM Method E1191; ASTM, 2001b). Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296, Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.03.005.pdf	Last Accessed:

Fact Sheet No.	2.1.3-11
Method Title	Chronic Echinoderm Fertilization Test, NHEERL-AED SOP 1.03.006
Purpose	These methods describe a toxicity test used to measure chronic effects from effluent and receiving waters to the gametes of the sea urchin, *Arbacia punctulata,* during a 48 hour exposure.
Method Summary	*Arbacia punctulata* are exposed to various effluent test concentrations that should be based on the objectives of the study. A dilution factor of 0.5 is used with this procedure, starting with a high concentration of 70% effluent . If the effluent is known or suspected to be highly toxic, a lower range of effluent concentrations should be used. Four females and four males are placed in shallow bowls, barely covering the animals with seawater. Both females and males will be stimulated to release their respective eggs or sperm. The egg stock and sperm are collected. Sperm are diluted and mixed with seawater. This sperm suspension is then distributed to vials and the number of sperm/mL are determined. The eggs are washed, diluted and counted. The test begins when diluted sperm are added to each test vial containing eggs and various dilutions of the effluent. All test vials are incubated for one hour at 20 °C. The suspension is then mixed and incubated again for 20 minutes. Fertilization is then determined using a Sedgwick-Rafter counting chamber. Fertilization is indicated by the presence of a fertilization membrane surrounding the egg. Larval development may also be measured in this test. The egg suspension is mixed and incubated for a longer period of time: 48 hours at 20 °C. At the termination of the test, the total number of larvae and the appropriately developed larvae are counted to determine survival and development per treatment. A similar toxicity test is described in the EPA guidance pertaining to effluents and receiving waters (Weber, 1991) and in Environment Canada's method EPS 1/RM/27.
Data Uses/Application	This sperm cell toxicity test determines the concentration of a test substance that reduces fertilization of exposed gametes relative to that of the control. This test may also be modified and used to assess pore water toxicity once the pore water is extracted from whole sediments.
Advantages	Sea urchin toxicity tests have been proven to be extremely sensitive indications of toxicity effects. The pore water toxicity tests with gametes and embryos of sea urchins are approximately an order -of-magnitude more sensitive than the 10-day solid-phase test with amphipods (Carr 2001).

Fact Sheet No.	2.1.3-11 (contd.)	
Limitations	Sea urchin toxicity tests are not considered standard toxicity tests, therefore fewer laboratories currently perform the test. Sea urchin tests have a limited salinity regime; therefore, they will be useful for samples from a marine environments and select estuarine environments. Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296. Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.03.006.pdf	Last Accessed:

Fact Sheet No.	2.1.3-12	
Method Title	Chronic Marine Fish Bioassay, NHEERL-AED SOP 1.03.004	
Purpose	This method describes a 7-day, static renewal chronic aqueous toxicity testing using the marine fish species, *Menidia beryllina* and *Cyprinodon variegatus*.	
Method Summary	The fish species, *Menidia beryllina* and *Cyprinodon variegatus*, are exposed to various concentrations of effluent over a 7-day period in 1-L test containers. The media concentration levels span a range of those causing zero mortality to those causing complete mortality. To determine effluent concentrations, one of two dilution factors is commonly used: approximately 0.3 or 0.5. *Menidia beryllina* and *Cyprinodon variegatus* are near coastal fish and are generally used in salinities greater than 25 ppt. Test temperature is $25^{o} \pm 2^{o}C$. The fish are fed daily. New dilutions of test media are prepared daily. The old solution is drawn out with a siphon and the newly prepared solutions are added to each chamber. The endpoints are survival and growth. Range-finding tests may be performed prior to these analyses to determine the appropriate test media dilutions. Reference toxicant tests will also be performed simultaneous to these tests. NPDES Guidance also outlines a 9-day static-renewal test with *Cyprinodon variegatus* to determine effluent effects on embryo larval survival and teratogenicity (Weber, 1991).	
Data Uses/Application	The results of this test will be used to determine the No Observable Effect Concentration (NOEC), Lowest Observable Effect Concentration (LOEC) and the Chronic Value (CHV). These results will determine the long term effects of those sediment samples on the surrounding biotic community.	
Advantages	Both fish species are considered benchmark species indicating that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species (USEPA and USACE, 1998).	
Limitations	Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296, Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.03.004.pdf	Last Accessed:

Fact Sheet No.	2.1.3-13	
Method Title	Toxicity Evaluations of Photoinduction of Polycyclic Aromatic Hydrocarbons (PAH): *In Situ* Analysis	
Purpose	This method was designed to evaluate the degree of photoinduced toxicity during wet-weather events via *in situ* experiments.	
Method Summary	*In situ* chambers are constructed with two long rectangular windows to allow water flow (holding 200 mL of water) and UV exposure (about 70% UV penetration) to organisms inside the chambers. Chambers containing *Ceriodaphnia dubia* are placed on the sediment surface in the shade or in the sunlight (four replicates each) at both a reference site and a test site. Dark mesh screens are placed over chambers on the shaded devices to further block out UV wavelengths. The chambers are retrieved 48-hours after being placed in the river. At test termination, the chambers are placed in coolers with site water and transported to the laboratory. Percent survival is determined within 6 hours of chamber collection. Water quality analysis is conducted at both the reference site and the test sites. Ultraviolet measurements are made at the surface and the bottom of the river. Water is collected also at the surface and the bottom in 1-L amber polyethylene sample bottles for PAH analysis. Similar freshwater crustacean species such as *Daphnia magna* and *Daphnia pulex* may also be used for similar assays.	
Data Uses/Application	The results of this *in situ* evaluation are analyzed to determine the acute toxicity of photoinduced PAHs. In particular, these tests are helpful in determine whether PAH effects are more prevalent after major storm events in both agricultural and urban environments.	
Advantages	*In situ* toxicity testing reduces sampling and laboratory-related errors from the assessment process. Fluctuating field conditions that may affect organism response cannot be mimicked in the laboratory. These organisms are benchmark species indicating that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species (USEPA and USACE, 1998).	
Limitations	Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	Ireland, D.S., Burton, G.A. and G.G. Hess. 1996. *In Situ* Toxicity Evaluations of Turbidity and Photoinduction of Polycylic Aromatic Hydrocarbons. Environmental Toxicology and Chemistry. Vol. 15: 4. p 574-581.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.1.3-14	
Method Title	Toxicity Evaluations of Photoinduction of Polycyclic Aromatic Hydrocarbons: Laboratory Analysis of Storm water	
Purpose	This method was designed to evaluate the degree of photoinduced toxicity during wet-weather events with *in situ* toxicity testing.	
Method Summary	Storm water runoff samples are collected in the field, returned to the laboratory and fractionated to measure toxicity. After an increase in toxicity is observed in the presence of UV radiation, a modified Toxicity Identification Evaluation procedure is performed via the following protocol (no standard protocol exists for a TIE with Storm water). Suspended solids are removed using glass fiber filters and the runoff is also filtered through B and J Solid Phase Extraction™ to remove all organics. With some modifications, the 7-day *Ceriodaphnia dubia* chronic toxicity test follows the U.S. Environmental Protection Agency's standard methods. In each 30 mL beaker, 25 mL of sample is placed in various dilutions intended to span a range of those causing zero mortality to those causing complete mortality. One organism is placed in each chamber. Water is renewed on days 3, 5, and 7. Organisms are fed daily. These tests are conducted in the presence of UV radiation. Lamps are constructed to emit amounts of visible and UV radiation, by using two cool-white fluorescent lamps: a 350-nm and a 300-nm photreactor lamp. Survival, reproduction and PAH concentrations are determined at the end of the 7-day test period.	
Data Uses/Application	The results of this evaluation are analyzed to determine the acute toxicity of photoinduced PAHS. In particular, these tests are helpful in determine whether PAH effects are more prevalent after major storm events in both agricultural and urban environments.	
Advantages	Laboratory tests, in comparison with *in situ* tests, can control other parameters such as temperature, pH etc in order to isolate effects as a result of contaminant levels. These organisms are benchmark species indicating that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species (USEPA and USACE, 1998).	
Limitations	Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	Ireland, D.S., Burton, G.A. and G.G. Hess. 1996. *In Situ* Toxicity Evaluations of Turbidity and Photoinduction of Polycylic Aromatic Hydrocarbons. Environmental Toxicology and Chemistry. Vol. 15: 4. p 574-581.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.1.3-15
Method Title	Growth and Scope for Growth Measurements with *Mytilus edulis*, NHEERL-AED SOP 1.03.013
Purpose	This method describes a test used to determine growth and the scope for growth (SFG) index using the blue mussel, *Mytilus edulis*. To derive the SFG index, the test measures the mussel's clearance rate, respiration rate and assimilation efficiency throughout the procedure.
Method Summary	Mussels collected in different field conditions are sorted by size and placed in individual clearance rate chambers. In these chambers, they are allowed to feed overnight on algae pumping through the system at a set concentration. A Coulter Counter is used to measure particles in order to determine the clearance rate. The mussels are moved from the clearance rate chambers to chambers where respiration measurement tools are set up. The respiration rate is measured in these chambers with a radiometer and 450 mL glass respirometer vessels, which are placed in the chambers with the individual mussels. Assimilation efficiency is measured in the clearance rate chambers after the chambers have been cleaned of fecal and algal matter and the mussels have feed overnight. Fecal pellets are collected to determine the dry weight and ash weight of feces. A similar procedure is completed with the cultured algae to obtain the dry weight and ash weight of the food. Mussel growth from pre exposure to post exposure is also determined using a vernier caliper. Lastly, the mussel tissues are excised, dried and weighed.
Data Uses/Application	The above method measures the following three physiological parameters in mussels collected from different field conditions: clearance rate, respiration rate and food assimilation efficiency. Clearance rate and assimilation efficiency measurements are used to determine total amount of energy available, while respiration rate is used to estimate metabolic energy costs. These data are used to calculate the SFG index, which is a measure of the energy available to an organism for somatic and reproductive growth after accounting for routine metabolic costs. Mussels of similar physiological condition should demonstrate similar SFG responses under standardized conditions; therefore, differences in SFG are attributed to persistent physiological effects of field exposure.

Fact Sheet No.	2.1.3-15 (contd.)	
Advantages	Investigators have found reduced growth and ultimately reduced fecundity and fitness in *Mytilus edulis* after sustained reduction in SFG. The SFG index, therefore, provides an additional way in which to quantify potential chronic effects from changes in field conditions. *Mytilus edulis* can withstand salinities ranging from 1 ppt to > 25 ppt. *Mytilis* is also considered a benchmark species indicating that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species (USEPA and USACE, 1998).	
Limitations	Please note the list of general limitations for all liquid-phase toxicity tests in the introduction to this section.	
Reference	USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296, Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.03.013.pdf	Last Accessed:

Fact Sheet No.	2.1.3-16	
Method Title	Microtox® tests, NHEERL-AED SOP 1.03.009	
Purpose	This method determines acute toxicity in effluents, receiving waters and elutriates through use of a bioluminescent bacteria. These tests are also applicable to whole-phase sediment samples.	
Method Summary	Microtox® tests measure acute toxic effects in luminescent bacteria (*Photobacterium phosporeum*) after exposure to effluents, receiving waters or elutriate samples (aqueous phase of a 4:1 water to sediment mixture). Metabolic inhibition in the luminescent organisms occurs if a sample is toxic, and the subsequent reductions in light output are used to derive a dose-response curve from which the effective concentration of the sample is determined. Water samples are evaluated at 45, 22.5, 11.3, and 5.6 % dilutions of the full-strength samples. The Microtox® reagent (*Photobacterium phosphreum*) are placed in the Microtox® turrets to measure initial light levels. The reagent is then added to the respective dilutions. Generally, the reagent reacts quickly to organic compounds and toxicity is elicited within 5 minutes. Metals take longer to elicit toxicity; up to 15 minutes should be allowed. After a set exposure time, the reagent and dilution are placed in a turret of the Microtox machine and the light levels are recorded. The Microtox® test is also conducted with undiluted samples (*e.g.* 100% test media).	
Data Uses/Application	Microtox® tests can be used to determine if the toxicity of liquid or solid phase samples extracted from a contaminated environment. They can either be used alone or in concert with other analyses screening samples as a preliminary step to identifying potential contamination.	
Advantages	Microtox® tests are much quicker than traditional toxicity tests and they may be performed in a field laboratory. They can be performed with liquid phase samples from both freshwater and marine environments. The apparent toxicity of elutriates can be a function of extraction solvent and overall procedure.	
Limitations	Bacteria response to potential toxicity in water or sediment may not be representative of the response of a larger organism encountering the same medium in the wild.	
Reference	USEPA. 1993a. Biological and Chemical Assessment of Contaminated Great Lakes Sediment, EPA 905-R93-006. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.03.009.pdf	Last Accessed:

Fact Sheet No.	2.1.3-17
Method Title	Comparative Toxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin to Seven Freshwater Fish Species During Early Life-Stage Development
Purpose	To determine and compare the toxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) to early life stages of freshwater fish on the basis of waterborne exposure of fertilized eggs.
Method Summary	Fish species suitable for this method include northern pike, white sucker, lake herring, fathead minnow, channel catfish, medeka, and zebrafish. Eggs are obtained by stripping the adult fish and artificially fertilizing the eggs in clean water. The exposure system consists of three tanks, one egg control tank, one solvent control tank and one test egg tank. Solvent and TCDD are added to the tanks (minus the control tank) 30 minutes prior to the start of each egg exposure. The recirculating flow rate in the tanks is adjusted to approximately 80 mL/min for all tests. Eggs are checked for fungus daily during the incubation period and dead eggs are removed and recorded. Following the hatch, all organisms are released into clean-water tanks and observed daily for signs of TCDD toxicity, which include edema, hemorrhaging, head and spinal deformities, lethargy, loss of equilibrium, skin discoloration, and mortality. The concentrations of TCDD in the test tanks are also measured and recorded. GC/MS analysis is used to determine the spercific activity and radiopurity of TCDD concentrations at the beginning and end of each exposure. A similar method for early life-stage toxicity testing is described in Environment Canada Method EPS 1/RM/28, 1st and 2nd editions. Available from Environmental Protection Publications, Environmental Protection Service, Environment Canada, Ottawa, Ontario, K1A OH3, Canada.
Data Uses/Application	TCDD is the most toxic of the hydrophobic, halogenated aromatic comounds that include polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). In addition, the toxic effects of TCDD are bioaccumulatable in aquatic systems. Because of its association with aquatic sediments, TCDD poses a potential risk to aquatic organisms. This method describes the determination of toxicity in the early-life stages of several fish species.
Advantages	For many fish species, the toxicity of TCDD is increased when the eggs are exposed prior to the hatch. The mechanism involved in TCDD uptake is known to be extremely functional in the very early life stages of fish and results of toxicity (other than mortality) are measurable in the post-hatch population.
Limitations	Comparisons of TCDD toxicity using this method to previously tested fish species are difficult because test conditions (exposure regimes and life stages) may vary so much. Also, within a fish species, sensitivity to TCDD is dependant on the age and size of the organism, and the exposure time and stage of development.

Fact Sheet No.	2.1.3-17 (cont'd)	
Reference	Elonen, Gregory E., RL Spehar, GW Holcombe, RD Johnson, JD Fernandez, RJ Erickson, JE Tietge, and PM Cook. 1998. Comparative Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin to Seven Freshwater Fish Species During Early Life-Stage Development. Environmental Toxicolgy and Chemistry, Vol. 17, No. 3, pp 472-483.	
Website	N/A	Last Accessed:

2.2 Sediments

Sediment samples are collected at Superfund sites with the same objectives in mind as when collecting water samples. These objectives, as stated in Section 2.1, are the following:

- To determine if the contaminant is hazardous by identifying its composition and characteristics;
- To determine if there is an imminent or substantial threat to public health or welfare or to the environment;
- To determine the need for long-term action;
- To develop containment and control strategies;
- To evaluate appropriate disposal/treatment options; and,
- To verify treatment goals or clean up levels (USEPA, 1994a).

To adequately characterize a site, the plans for sediment sampling and related analyses must be developed in consideration of the site characteristics. Therefore, the following fact sheets relating to sediment are divided into sections pertaining to field sample collection and processing, chemical and physical analyses, and biological analyses. These fact sheets intend to provide Superfund managers with a summary of the existing methods that may be applicable to their site, the method's relative strengths, and the method's relative weaknesses.

2.2.1 Field Sample Collection and Processing, *In Situ* Data Acquisition

Section 2.2.1 provide field sample collection and processing methods for sediments. Sediments are collected at Superfund sites for sediment chemistry, toxicity and benthic community analyses to determine the extent of chemical contamination and impact of contamination on the site . The two primary methods for sediment collection include sediment grab samplers and sediment core samplers. However, there are quite a few different types of grabs and cores. These different samplers are summarized following their respective fact sheets in Tables 2.2.1-1 and 2.2.1-2. Other sediment and processing collection methods are also provided for situations where grab and core deployment is un necessary or impossible due to physical interferences. These sample collection methods were gathered from the following information sources:

- The USEPA's Office of Water
- The USEPA's Office of Research and Development
- ASTM
- Standard Methods for Examination of Water and Wastewater, 1999
- Puget Sound Water Quality Action Team
- The USEPA's Coastal EMAP Program
- The USEPA's Great Lakes Program Office

Field observations and preliminary identification of sediment type are pertinent to all sediment collections. Guidelines for making visual observations of sediment type can be found in the US Army Corps of Engineers manual on Soil Sampling, EM 1110-1-1906.

Fact Sheet No.	2.2.1-1
Method Title	Grab Sampling
Purpose	To collect samples of the benthos for quantitative or qualitative sampling procedures, intended to determine sediment chemistry, toxicity and/or benthic community composition and abundance.
Method Summary	Grabs are to be lowered slowly from a boat or by hand into the water column. When most grabs reach the bottom their weight will cause them to penetrate the substrate (areas of 0.02 to 0.5 m^2 and depths ranging from 5 to 15 cm). The slack on the cable allows the locking lever to release, therefore permitting the movement that allows the horizontal locking bar to drop out of the locking notch and allow the jaws to close as the device is raised. Other grabs are closed by spring action or some other mechanical device after penetrating the substrate. After the grabs are brought to the surface, they are examined for acceptability. Collection of undisturbed sediment requires that the sampler : -create a minimal pressure wave when descending -form a leakproof seal when the sediment sample is taken -prevent winnowing and excessive sample disturbance when ascending -allow easy access to the sample surface in order that undisturbed subsamples may be taken (USEPA, 1992c). The required amount of sediment is removed for sub-sampling and placed in the appropriately cleaned sample containers. (See Fact Sheet 2.2.1-8.) Other USEPA, ASTM and APHA documents provide grab sample collection information and details pertaining to different grab samplers (USEPA, 1992c; ASTM Method E1391 (ASTM 2001b); Standard Method 10500 (APHA 1999). There are many types of grabs that vary in penetration depth, surface area sample and sampling various substrate types. The various grabs and their respective advantages and disadvantages are detailed on the following matrix (Table 2.2.1-1).
Data Uses/Application	Grab sampling devices collect sediments that may be used to analyze for sediment chemistry, sediment toxicity, and/or the samples may be sieved to determine benthic community composition and abundance. They are commonly used in estuarine and marine monitoring programs due to their ability to provide reliable quantitative data at a relatively low cost.
Advantages	Since there are many types of grabs, it is easy to find one that will be effective in various site conditions (Table 2.2.1-1). Grabs are able to sample a larger surface area than most coring devices.

Fact Sheet No.	2.2.1-1 (contd.)	
Limitations	Grabs have a relatively shallow and variable depth of penetration depending on the sediment properties. As the grab sampler bites into the sediment, the sediment is inevitably folded resulting in the loss of information concerning the vertical structure of sediments. The shock wave that results from the grab's deployment also results in a loss of the fine surface sediments and water-soluble compounds and volatile organic compounds present on the surface of the sediment.	
Reference	USEPA. 1990b. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/bioiweb1/pdf/benthos_methods_ch5.pdf	Last Accessed: 1/28/2003

Table 2.2.1-1. A Summary of Sediment Grab Devices (USEPA, 1992c; APHA, 1999; USEPA, 1990b; Murdoch and Azcue, 1995)

Sediment Grab	Habitat	Substrate Type, Surface Area and Penetration Depth	Advantages	Disadvantages
Van Veen	Open sea and large lakes	Sand, silt, clay or similar substrates Surface Area: 0.25 m^2 Penetration Depth: 5-7 cm	This grab can sample most sediment types. Large enough to permit sub-sampling.	Shock wave from descent may disturb "fines." Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.
Young Grab (fluorocarbon plastic or kynar lined modified 0.1 m^2 van veen)	Lakes and marine areas	Sand, silt, clay or similar substrates Surface Area: .1 m^2	Lined grabs eliminate metal contamination. Small size reduces pressure wave.	Expensive, requires winch.
Orange Peel	Marine environments and deep lakes	Sandy substrates Round grab; collects up to 1600 cm^3 of sediment	Comes in a range of sizes, works in deep water, closes relatively well to prevent sample loss, good for reconnaissance.	Very heavy, requires power winch. Does not sample constant area and depth.
Smith McIntyre	Marine, estuarine, adaptable to large rivers, lakes and reservoirs	Used on most substrates; designed specifically to sample hard substrates Surface Area: 0.2 or 0.1m^2	This grab is stable and easy to control in rough water.	Loss of fines. Possible contamination from metal frame construction. Very heavy, requires a power winch.
Shipek	Used primarily in marine waters and large inland lakes and reservoirs	Sand, gravel, mud and clay Surface Area: 0.4m^2 Penetration Depth: 10 cm	This grab is good for collecting a small sample in deep water. It has a sample bucket from which a sub-sample may be obtained. It retains fine-grained sediments effectively.	Possible contamination from metal frame construction. Very heavy, requires a power winch.
Petersen	Freshwater lakes, reservoirs, rivers and estuaries	Useful on most substrates; especially hard substrates with swift currents and deep water.	This grab can obtain a large sample and it can penetrate most substrate.	Heavy, may require winch. No cover lid to permit subsampling. All other disadvantages of Ekman and Ponar.

Table 2.2.1-1. (contd.)

Petite Ponar	Deep rivers, lakes and reservoirs	Moderately hard sediments, such as sand, silt and mud. Surface Area: .02 m^2	This grab has a good penetration for a small grab, side plates and screens to prevent washout, and it can be operated by hand without a boat or a winch.	It is not effective for sampling deep burrowing organisms or for sampling clay substrate.
Ponar	Deep rivers, lakes and reservoirs	Fine to coarse textured sediments such as clay, hard pan, sand, gravel and muck. Less effective in softer sediments. Surface Area: .05 m^2	This grab is very efficient for hard sediments; considered universal sampler due to ability to collect adequate samples from most substrate types.	Shock wave from descent may disturb "fines." Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.
Ekman or Box Dredge	Can be used from boat, bridge, or pier in waters of various depths	Consolidated, fine textured sediments. Efficient in soft sediments, such as silt, muck and sludge in water with little current. Surface Area: .05 m^2	This grab is light weight; it can be operated by hand. It is commonly used for benthic evaluations. Obtains a larger sample than coring tubes. Can be subsampled through box lid.	Shock wave from descent may disturb "fines." Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis. Difficult to use in rocky or sandy bottoms

Fact Sheet No.	2.2.1-2
Method Title	Core Samplers
Purpose	To collect an undisturbed sediment sample from varying depths in any substrate.
Method Summary	Prior to deployment, the sampling device is inspected to see that the sediment retainer behind the cutting edge will provide a good seal. For a box corer, the cable must feed through the pulley system properly and the spade must rotate freely. All portions of the sampling device that will be in contact with the sample (*i.e.,* the core tube and the core liner, where applicable) should be constructed of noncontaminating material. Cores are deployed from a suitable vessel. Cores use either inertia (*i.e.,* gravity cores, piston cores) or mechanical motion (hammering or vibration) as the primary driving force to achieve the desired penetration depth depending on the specified depth and the sediment properties. The amount of pull that is required to extract a core tube from the substrate depends on the specific gravity of the device and its contents, plus the amount of frictional force against the surface of the core tube walls that must be overcome. During the extraction, the wire strain should be steady and continuous; the vessel should be held stationary directly above the coring device. Once clear of the bottom, winch take-up speed may increase. Once the sampling device is onboard the vessel, one or both ends of the core tube is capped if possible. Overlying water is siphoned off at the top of the core tube (after allowing for settling time). The length of the sediment core should be determined by comparing measurements of the length of the core material against the overall penetration depth. The ratio of penetration depth to core material length is calculated to determine the compaction of the sediment during coring. If the core is acceptable (for example, acceptable depth of penetration, surface layer intact), the core tube (or liner) should be labeled with the core identification number, collection date, core orientation, and length of core material collected. Until the core sample can be extruded or split into sections, the core tube should be secured in an upright position, taking care not to avert the core. Cores should be split within 24 hours of collection. Other USEPA, ASTM and APHA method documents provide specifics regarding core sampling and determining what type of core device is applicable (USEPA, 1990b; ASTM Method E1391(ASTM 2001b; Standard Method 10500 (APHA, 1999). There are also several types of cores; these cores and their respective advantages and disadvantages are detailed on the following table (Table 2.2.1-2).

Fact Sheet No.	2.2.1-2 (contd.)	
Data Uses/Application	Core samples can be analyzed for sediment chemistry, sediment toxicity and/or benthic community analyses.	
Advantages	Core sampling removes sediments with less disruption than grab and dredge sampling. Gravity corers or hand-driven corers can collect sediments up to 1 to 2 m in depth. When vibratory corers or others using hydralics for sediment penetration, corers can collect sediments up to 10 m in depth. Corers are more efficient than grabs. Corers are the most accurate samplers of benthic macroinvertebrate populations.	
Limitations	Core sampling provides an imprecise estimate of the standing crop of macrobenthos, because of the small area sampled. Gravity operated samples have limited surface area, and they require a boat and powered winch. Cores generally sample an area 13 to 26 cm^2. Cores are more difficult to handle than grabs in rough water. They do not work well in sandy sediments.	
Reference	PSWQAT. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound, Puget Sound Protocols and Guidelines. Puget Sound Water Quality Action Team, Olympia, WA.	
Website	http://www.psat.wa.gov/Publications/protocols/protocol_pdfs/field.pdf	Last Accessed: 1/28/2003

Table 2.2.1-2. A Summary of Sediment Coring Devices (USEPA, 1992c; APHA, 1999; USEPA, 1990b; Murdoch and Azcue, 1995)

Sediment Core	Core liner diameter; Penetration Depth	Substrate Type; Habitat	Advantages	Disadvantages
Fluorocarbon plastic or Glass tube	Core diameter: Varies Penetration Depth: Up to 50 cm	Shallow wadeable waters or deep waters if SCUBA available	Preserves layering and permits historical study of sediment deposition. Minimal risk of contamination.	Small sample size requires repetitive sampling.
Hand Corer	Core diameter: 3.5 to 7.5 cm ID Penetration Depth: 50 to 120 cm	Soft or semi-compacted sediment in shallow wadeable waters; deep waters if SCUBA available	Handles provide for greater ease of substrate penetration. Preserves layering and permits historical study of sediment deposition. Minimal risk of contamination.	Small sample size requires repetitive sampling. Careful handling necessary to prevent spillage. Requires removal of liners before repetitive sampling. Slight risk of metal contamination from barrel and core cutter.
Push Core	Varies	Soft or semi-compacted sediment in shallow water	Push cores with shallow penetration and in relatively shallow water do not require a winch.	They are difficult to deploy in areas with strong currents and deep water. They may be difficult to retrieve if the penetration depth exceeds 50 cm.
Phleger Core (Gravity Core)	Core diameter: 3.5 cm ID Penetration Depth: Up to 50 cm	Soft substrates, semi-compacted substrates, peat and vegetated roots in shallow lakes and marshes	There is low risk of undisturbed sample contamination. This sampler maintains sample integrity relatively well. It does not require a winch.	Careful handling is necessary to avoid sediment spillage. Small sample, requires repetitive operation and removal of liners. Time consuming.
KB Core (Gravity Core)	Core diameter: 3.5 cm ID and 5 cm Penetration Depth: Up to 70 cm	Soft, fine-grained substrates	Useful for obtaining estimates of the standing crop of macrobenthos in soft substrates. Winch is not required.	The messenger system used to close the core is sometimes ineffective when the core does not penetrate the sediment vertically.
Box Core (Gravity Core)	Surface area: typically 0.04 and 0.1 m^2 Penetration Depth: Up to 1 m	Soft sediments	This core samples the same surface area as a grab, but it disturbs the surface less. Allows for subsampling.	This core does not penetrate sediments deeply. Hard to handle, very heavy.

Table 2.2.1-2. (contd.)

Benthos Gravity Core	Core diameter: 6.6 cm and 7.1 cm Penetration Depth: Up to 3 m	Soft, fine-grained substrates	It has stabilizing fins that promote vertical penetration. It also has a valve system that prevents sample loss. It can sample substrates from great depths.	More difficult to deploy and retrieve, large device, very heavy.
Alpine Gravity Core	Core diameter: 3.5 cm Penetration depth: 0.6, 1.2 and 1.8 m	Compacted substrates from depth	It can sample substrates from great depths.	Lack of stabilizing fins makes vertical penetration difficult. This core also disturbs surface sediments significantly. Very heavy.
Multi-Gravity Core	Core diameter and penetration depth vary.	Varies	Multiple samples can be taken from one site for comparative studies, evaluation of sediment samples and determination of sediment heterogeneity over a small area.	Large and difficult to deploy and retrieve.
Piston Core	Core diameter: typically 3, 5, or 6" Penetration depth: 3-20 meters	Soft substrates	This core can be used for samples requiring significant penetration depths. Relatively undisturbed samples	A heavy crane is needed with lifting capacity over 2000kg to deploy and retrieve piston cores. This device is not suitable for sediment profiles since it disturbs the top layer.
Vibratory-Hammer Core	Core diameter: typically 3, 5, or 6"	All types	This core can be used in all types of sediments. Method of choice for many environmental dredging studies.	Large and difficult to deploy and retrieve.

Fact Sheet No.	2.2.1-3	
Method Title	Hand Collection	
Purpose	This method describes how sediment samples can be collected by hand in the intertidal zone with a favorable tide.	
Method Summary	Sediment samples may be collected by hand with a variety of sampling implements such as spoons or trowels for surface sediments, or with hand augers or corers for collecting sediments at discrete depths. Any sampling implement that comes in contact with the sample should be constructed of stainless steel or Teflon™. If individual sample collection kits are not available for each sampling location, sampling equipment should be thoroughly decontaminated between stations by scrubbing with a phosphate-free detergent solution, followed with a thorough rinse with analyte-free water. If heavy contamination by metals or organic contaminants is expected at the site, sampling equipment may be rinsed with methanol, acetone or a 50:50 acetone/hexane mix for organics or 10% HNO_3 for metals. Once the samples have been collected with one of the aforementioned tools, the samples should be homogenized in a stainless steel bowl with a stainless steel or Teflon™ spoon or spatula. Sample aliquots are transferred to appropriate laboratory supplied containers and preserved as required.	
Data Uses/Application	These methods are used in shallow waters where a core is not needed. Sediment samples can be analyzed for sediment chemistry, sediment toxicity and/or benthic community analyses.	
Advantages	Hand collection is less expensive and labor intensive than other sediment collection techniques.	
Limitations	Sediments can only be collected by hand in shallow waters or locations where the tide has exposed the desired sampling area. This collection procedure also increases human exposure to potential contaminants present in the samples.	
Reference	PSWQAT. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound, Puget Sound Protocols and Guidelines. Puget Sound Water Quality Action Team, Olympia, WA.	
Website	http://www.psat.wa.gov/Publications/protocols/protocol_pdfs/field.pdf	Last Accessed: 1/28/2003

Fact Sheet No.	2.2.1-4	
Method Title	Hand Collection at Depth with SCUBA Equipment	
Purpose	To sample benthos in locations where conventional sampling devices are not practical and the water depth is too deep for hand-collection.	
Method Summary	The collection of sediment samples by a diver should be considered when undisturbed samples are required, particularly for studies of the sediment-water interface. SCUBA certified professionals can conduct the following types of benthos sampling: placement and retrieval of artificial substrate; use of suction samplers; sampling with a quadrate frame; and , perhaps most importantly, identifying and delineating substrate types for purposes of determining sampling effort (stratified sampling) and choice of samplers.	
Data Uses/Application	SCUBA divers can use traditional sediment collection devices that are then analyzed for sediment chemistry, sediment toxicity, and/or the samples may be sieved to determine benthic community composition and abundance.	
Advantages	Allows for benthic sampling in locations that are inaccessible by conventional grab sampling. Allows for more precise sampling of the sediment-water interface.	
Limitations	Requires certified SCUBA divers who will comply with rigid safety standards. Somewhat expensive. The diver's visibility can be obscured if fine-grained sediments are disturbed or if the water is turbid.	
Reference	USEPA. 1990b. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/bioiweb1/pdf/benthos_methods_ch5.pdf	Last Accessed: 1/28/2003

Fact Sheet No.	2.2.1-5	
Method Title	Sediment Traps	
Purpose	Sediment traps collect settling particulate matter (SPM) which provides useful data for studies of sedimentation rates and resuspension of bottom sediments.	
Method Summary	If collecting SPM for chemical analysis, the traps should be cleaned with a phosphate-free detergent solution, then sequentially rinsed with hot water, 10 percent HNO_3, analyte-free, pesticide grade acetone, and finally, wrapped in aluminum foil until deployment in the field. If the sediment trap is constructed of plexiglass, the acetone rinse should be avoided as acetone will damage the plexiglass. Prior to deployment, the traps should be filled with two liters of high-salinity, analyte-free water containing a preservative to reduce microbial degradation of the samples during deployment period. SPM samples are collected by retrieving the traps and removing the overlying water in the collection cylinders using a peristalic pump. The water immediately overlying the trapped sediment is analyzed to determine the salinity and the presence of preservative to determine if the trap was disturbed during the deployment. The SPM is then transferred to sample containers and taken to an analytical laboratory for processing. The particulate fraction of the SPM is removed by centrifuge and split into sample aliquots for chemical analysis. Sediment trap designs vary. The trap must be made suitable for the study area. Considerations on biofouling must be addressed and controlled appropriately with regard to the type of samples being collected.	
Data Uses/Application	SPM data are used for studies of sedimentation rates and for sediment transport studies.	
Advantages	Sediment traps can collect relatively large volumes of suspended matter for transport studies (compared to filtration).	
Limitations	Construction, deployment, and retrieval often require resources beyond typical field studies. Biological invasions of traps typically occur. Controlling agents are not suitable for all applications.	
Reference	PSWQAT. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound, Puget Sound Protocols and Guidelines. Puget Sound Water Quality Action Team, Olympia, WA.	
Website	http://www.psat.wa.gov/Publications/protocols/protocol_pdfs/field.pdf	Last Accessed: 1/28/2003

Fact Sheet No.	2.2.1-6	
Method Title	Russian Peat Borer	
Purpose	To collect discrete, relatively uncompressed sediment samples.	
Method Summary	The Russian Peat Borer is a manually driven, chambered-type, side-filling core sampler, and its components include a stainless-steel core tube, aluminum extension rods, a stainless-steel turning handle, and a Delrin ® core head and bottom point that support a stainless-steel cover plate. To collect a sediment sample, the bottom point of the Russian Peat Borer is manually inserted into sediment, with the blunt edge of the core tube turned against the cover plate to prevent sediment from entering the tube during penetration. A slide-hammer mechanism can be used to drive the sampler through highly consolidated sediment or peat that is hard to penetrate. Once the sampler is driven into the sediment to the desired depth, the core tube is turned 180 degrees clockwise. This allows the core tube to rotate and its sharp edge to longitudinally cut through the sediment, collecting a semicylindrical sediment core. While the core tube is manually turned, the stainless-steel cover plate provides support so that the collected material is retained in the core tube. The sampler is then rotated and placed on the sampling platform in such a way that the core tube is above the cover plate. The core tube is then manually turned counterclockwise, rotating the tube and exposing the semicylindrical core sample on the cover plate.	
Data Uses/Application	The sampler can collect discrete, relatively uncompresssed core samples from shallow to deep depth intervals with out disturbing the sediment stratification.	
Advantages	The sampler is lightweight and easy to operate, requiring minimal training and skill. It does not require disassembly to extrude the sample. It requires no support equipment other than two sawhorses for support during sample extrusion.	
Limitations	For deployment in deep water applications, the sampler requires extension rods. This sampler is not equipped with disposable core liners. During deployment, the cover plate is exposed to different layers of sediment. Also, partially decomposed plant matter or small stones may cause the core tube to remain in the open position during sampling retrieval. To use this sampler, sediment must offer enough support to keep the cover plate stationary and allow rotation of the tube core.	
Reference	USEPA. 1999d. Innovative Technology Verification Report: Aquatic Research Instruments Russian Peat Borer, EPA/600/R-01/010. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/ordntrnt/ORD/SITE/reports/600r01010.htm	Last Accessed: 1/28/2003

Fact Sheet No.	2.2.1-7	
Method Title	Split Core Sampler for Submerged Sediments	
Purpose	To collect undisturbed core samples of sediment up to a maximum depth of four feet below sediment surface.	
Method Summary	The fully assembled sampler is manually lowered into the water in such a way that the coring tip is placed on the sediment surface. The sampler can then be either manually pushed with the cross handle or driven with the slide-hammer or an electric hammer to the desired sediment depth. The sampler is removed from the sediment either manually by reverse hammering or with the tripod-mounted winch. Once the sampler has been retrieved, either the interlocking split core tubes are disassembled or the coring tip or top cap is removed to allow removal of the core tube liner.	
Data Uses/Application	This sampler is designed to collect undisturbed, cylindrical core samples of various types of sediment, including saturated sands and silts, to a maximum depth of 48 inches below the sediment surface.	
Advantages	The sampler is lightweight and easy to operate, requiring minimal skills and training. An (SOP) accompanies the sampler when it is purchased. A combination of stainless-steel split core tubes can be used to collect 6- to 48-inch-long sediment cores. Plastic core tube liners can be used with the sampler. The sampler design uses a ball check valve-vented top cap. This feature: (1) allows air and water to exit the sampler during deployment, (2) prevents water from entering the sampler during retrieval, and (3) creates a vacuum to help retain a sediment core during sampler retrieval.	
Limitations	The core tube liner is exposed to different layers of sediment contamination during sample collection. The ball check valve-vented top cap may become clogged if the sampler is deployed in such a way that the top cap is below the sediment surface. The sampler cannot collect discrete samples from various sediment depths and is subject to core shortening. Because an external power source is required to operate the electric hammer, the sampling platform must be able to accommodate the weight and size of a portable generator. Use of the tripod-mounted winch or similar device limits the sampling platform locations from which the sampler can be deployed.	
Reference	USEPA. 1999e. Sediment Sampling Technology Art's Manufacturing and Supply, Inc., Split Core Sampler for Submerged Sediments, Superfund Innovative Technology, Evaluation Program, EPA/600/R-01/009. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/ORD/SITE/reports/600r01009.htm	Last Accessed: 1/28/2003

Fact Sheet No.	2.2.1-8	
Method Title	Sediment Processing for Chemistry and Toxicity Testing	
Purpose	These methods describe protocols for removing sediment samples from sampling collection devices and processing them for laboratory analyses.	
Method Summary	A clean stainless steel or Teflon™ spoon is used to remove sediments from grab samples for these analyses. Surficial sediments are removed (usually 0-2 cm) and placed in a stainless pot. The pot is then placed in a cooler on ice (not dry ice) and stored at 4°C. This process is repeated until sufficient quantity of sediment has been collected and composited with the other sediments (approximately 4 L). Sediments from sediment cores are extruded and subsampled also for the following analyses. For organic analysis, 250 cc of sediment is placed into a 500 mL pre-cleaned, glass bottle for chemical analysis. The sample number is recorded and the jar is wrapped in bubble wrap (to prevent breakage) and packed in ice. For metals, approximately 100 cc of sediment is placed into pre-cleaned, plastic (HDPE) sampling jars. The sample number is recorded and the sample is kept on ice at 4° C. For Total Organic Carbon, approximately 100 cc of sediment is placed into pre-cleaned, glass sampling jars. The sample number is recorded and the sample is kept on ice at 4° C. For sediment grain size, approximately 100 cc of sediment is placed into a clean plastic (HDPE) sampling jar or whirl-pak bag. The sample number is recorded and the sample is kept on ice at 4° C. For sediment toxicity, the volume of sediments collected will vary depending on the objectives and methods of the specific toxicity tests.	
Data Uses/Application	These methods describe the steps necessary to prepare samples for toxicological or chemical analyses. The applicability of these tests and analyses are described in their respective fact sheets (Sections 2.2.2 and 2.2.3).	
Advantages	Compositing the surface 2 cm of sediment from multiple grabs or cores allows a representative samples to be collected. This process provides sufficient sediment volume for toxicity testing and supporting chemistry and physical measurements.	
Limitations	Extreme care must be taken to ensure that the samples are not contaminated during sampling or processing procedures.	
Reference	USEPA. 2000b. Coastal 2000 Northeast Component: Field Operations Manual, Environmental Monitoring and Assessment Program (EMAP). EPA/620/R-00/002. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/emap/nca/html/docs/c2kneefm.pdf	Last Accessed: 1/28/2003

Fact Sheet No.	2.2.1-9	
Method Title	Sediment Processing for Elutriate Toxicity Tests	
Purpose	This method describes how elutriate samples are prepared for toxicity tests from the original whole-phase sediment samples.	
Method Summary	Elutriates are prepared with one part sediments (from sampling location) and four parts reconstituted dilution water. A 200 g sub-sample of homogenized sediment is removed from the containers and placed in a centrifuge bottle with 800 g of dilution water (usually site water). The contents are weighed, mixed and centrifuged. The overlying water is removed and the elutriate sample is sub-sampled and stored in 1-L amber bottles equipped with Teflon™-lined lids until testing. Test organisms are exposed to varying concentrations of the elutriate material (0%, 12.5 %, 25%, 50% and 100%) for a designated period of time	
Data Uses/Application	Elutriate toxicity tests provide information that can be used to support inferences about the potential toxicity of the contaminated sediments from which the elutriates are prepared and to identify the biologically active constituents of the contaminated sediment. Elutriate tests are commonly used to evaluate proposals to discharge dredged material into ocean waters, and to evaluate the potential of the dredged materials to impact ocean ecology.	
Advantages	Elutriate tests allow the investigator to assess the potential hazard of contaminated sediments to aquatic organisms, to compare the relative toxicity of contaminated sediments from different locations, and to study the biological availability of the contaminants associated with sediments.	
Limitations	Elutriate toxicity tests do not necessarily reflect the toxicity of in-place sediment.	
Reference	USEPA. 1993b. Assessment and Remediation of Contaminated Sediments (ARCS) Program: Biological and Chemical Assessment of Contaminated Great Lakes Sediment, EPA 905-R93-006. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.1-10	
Method Title	Sediment Processing for Pore Water Extraction through Centrifugation, E1391-02	
Purpose	This method describes a process for separation and collection of interstitial pore water from sediment samples to provide either a matrix for toxicity testing or an indication of the concentration and partitioning of contaminants within the sediment matrix.	
Method Summary	Centrifugation may be used to isolate interstitial water for chemical or toxicological analyses. The centrifugation conditions (*i.e.,* speed and temperature) will vary considerably depending on the contaminants potentially present in the pore water. Similarly, the filtration scheme will depend on the sediment composition and the analytes of interest. For dissolved metals and dissolved organic carbons, sediments should be centrifuged at high speed and filtered with a 0.2-um membrane filter. For other dissolved organic contaminants, collodial matter, and aquatic bacteria, sediments should be centrifuged at a lower speed and filtered through a 0.45-um membrane filter. Generally, 30 minutes of centrifugation at 10,000 x g is recommended for routine toxicity testing of interstitial waters. The temperature should be set to reflect ambient temperature at time of collection.	
Data Uses/Application	Interstitial water is analyzed to either provide a matrix for toxicity testing or an indication of the concentration and partitioning of contaminants within the sediment matrix. There is some indication that interstitial water may be as useful as whole sediment for evaluating the toxicity of some sediment-associated compounds.	
Advantages	Centrifugation will extract a relatively large volume of interstitial water as compared to other separation techniques.	
Limitations	Centrifugation procedures vary depending on the various compositions of the sediments, therefore it is difficult to have an established protocol. Manipulation and centrifugation changes the redox potential of the pore water from the *in situ* conditions. Filtration may also remove toxicity. Sometimes a double centrifugation is required to remove fine particles.	
Reference	ASTM. 2001b. ASTM Book of Standards. Section 11.05. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.1-11	
Method Title	Pore Water Extraction from Sediments through Squeezing, ASTM E1391-02	
Purpose	This method describes a process for separation and collection of interstitial pore water from sediment samples to provide either a matrix for toxicity testing or an indication of the concentration and partitioning of contaminants within the sediment matrix.	
Method Summary	The apparatus used for isolation of pore water by squeezing includes a filter. The characteristics of filters should be considered carefully based on the types of contaminants expected. An example method for squeezing can be found in Manheim (1966). Briefly, the apparatus consists of a standard laboratory press and a filter unit containing a stainless steel screen, perforated steel plate, steel filter holder and filters. Wet sediment is transferred into a cylinder at the top of the apparatus. The whole unit is placed in a press and pore water is removed through a bottom filter. The extraction time will vary depending on the amount of sediment placed in the unit.	
Data Uses/Application	Squeezing is used to extract pore water from loose seabed sediments. Interstitial water is analyzed to either provide a matrix for toxicity testing or an indication of the concentration and partitioning of contaminants within the sediment matrix. There is some indication that interstitial water may be as useful as whole sediment for evaluating the toxicity of some sediment-associated compounds.	
Advantages	This is a rapid, reproducible method for pore water collection.	
Limitations	Squeezing can produce artifacts due to shifts in equilibrium from pressure, temperature, and gradient changes. It can also affect the electrolyte concentration and redox potential compared to *in situ* conditions. Filtrations may remove toxicity from water samples. It is difficult to choose a filtering scheme without knowing the cause of toxicity or the mixture of the toxicant.	
Reference	ASTM. 2001b. ASTM Book of Standards. Section 11.05. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.1-12	
Method Title	Pore water extraction from sediment from Vacuum Filtration, ASTM E1391-02	
Purpose	This method describes a process for separation and collection of interstitial pore water from sediment samples to provide either a matrix for toxicity testing or an indication of the concentration and partitioning of contaminants within the sediment matrix.	
Method Summary	Vacuum filtration is one of several methods (including gas pressurization and displacement) that can be used to remove pore water from sediments for chemical analysis when only a small volume is required. A vacuum extractor consists of a fused-glass air stone connected to a syringe with tubing. The syringe is then used to extract the water until sufficient volume has been collected.	
Data Uses/Application	Interstitial water is analyzed to either provide a matrix for toxicity testing or an indication of the concentration and partitioning of contaminants within the sediment matrix. There is some indication that interstitial water may be as useful as whole sediment for evaluating the toxicity of some sediment-associated compounds.	
Advantages	These methods are useful for collecting small amounts of water for chemical analysis.	
Limitations	Problems common to this type of method are a loss of equilibration between the interstitial water and the solids, filter clogging, and oxidation. Further research is needed to demonstrate the utility of this method to *in situ* collection of sediment pore water.	
Reference	ASTM. 2001b. ASTM Book of Standards. Section 11.05. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.1-13	
Method Title	Acoustic Sub-Bottom Profiling Systems, DRP-2-03	
Purpose	To collect sub-bottom data for sea-floor mapping.	
Method Summary	High-resolution acoustic profiling systems use high power signals to penetrate the sediments of the sea floor. The signal is reflected from interfaces between sediment strata of different acoustic impedance. These data are printed on a graphic recorder as a continuous two-dimensional profile. The amount of penetration will depend on the combination of the frequency and power of the profiler being used. A 3.5 - 14 kHz frequency pulse is typically used. Penetration also depends on the material type which composes the bottom and sub-bottom. For instance, differences in soil types, density, water content, and degree of solidification greatly influence the reflecting properties of the sub-bottom strata. Most Sub-bottom surveys are conducted in conjunction with bathymetric and/or side scan sonar surveys. The survey uses a predetermined grid pattern with lines spaced at variable distances, depending on objectives of the survey. Small survey boats (30 to 65 ft long) are adequate for performing a complete multi system survey. Over-the-side, surface-towed, and hull-mounted source/receiver arrays can be used. Data acquisition must be interfaced with the navigation system so that accurate information between position and data is recorded at all times. Environmental Effects of Dredging Technical Notes EEDP-01-5 and EEDP-01-10 also discuss Sub-bottom profilers (US Army Engineer Waterways Experiment Station, 1989; US Army Engineer Waterways Experiment Station, 1988). Sub-bottom profiling is also discussed in the *Guidelines for the conduct of benthic studies at aggregate dredging sites* by the U.K. Centre of Environment, Fisheries and Aquaculture, available online (www.planning.odpm.gov.UK/benthic Last accessed 2/10/03).	
Data Uses/Application	Typical applications include the monitoring of sediment disposal sites to detect stratification within and just below deposits.	
Advantages	Primary advantages associated with acoustic sub-bottom profiling are continuous documentation of reflecting strata, rapid coverage, and relatively low cost.	
Limitations	The quality of records obtained in seismic reflection studies depends greatly on the presence of subsurface horizons which will reflect acoustic energy. Records in deep water will tend to show average conditions over an area rather than a specific profile directly below the ship. Effective use of this instrumentation requires a trained operator.	
Reference	US Army Engineer Waterways Experiment Station. 1991. Hydrologic Surveys Applicable to Dredging Operations. DRP-2-03. Waterways Experiment Station, U.S. Army Corps of Engineers, Vicksburg MS.	
Website	http:/el.erdc.usarce.army.mil/elpubs/pdf/drp2-03.pdf	Last Accessed: 1/28/2003

Fact Sheet No.	2.2.1-14	
Method Title	Side Scan Sonar, EEDP-01-10	
Purpose	To qualitatively map the surface characteristics of the sea floor.	
Method Summary	During side scan sonar analysis, an acoustic towfish projects acoustic energy at a frequency of 100-500 kHz in a lateral direction using a pair of transducers. The acoustic signal bounces off of the sea floor back to the transducers on the towfish. The received signal is transmitted through the tow cable to the shipboard receiver, which processes the signal and produces a sonograph. A frequency of 100 or 500 kHz is generally used for monitoring disposal sites. The lower frequency gives a greater range (*i.e.,* 200-400 m of bottom compared to 100 m at 500 kHz) but provides less detail. At 500 kHz, the sonar is able to distinguish differences in bottom texture that can be used to map grain size. For example, low-backscatter indicates a silty bottom. Sand ripples in the image can be used to interpret grain-size variation and the movements of sediment. Environmental Effects of Dredging Technical Notes EEDP-01-5 briefly discusses side scan sonar (US Army Engineer Waterways Experiment Station, 1989). Side scan sonar is also discussed in the *Guidelines for the conduct of benthic studies at aggregate dredging sites* by the U.K. Centre of Environment, Fisheries and Aquaculture, available online (www.planning.odpm.gov.UK/benthic Last accessed 2/10/03).	
Data Uses/Application	Typical applications include the monitoring of sediment disposal sites before and after disposal. 300 kHz or higher frequencies can also be used for habitat mapping (*i.e.*, sea grass beds). The information gathered with side-scan sonar may be used to direct subsequent monitoring studies.	
Advantages	Overlapping coverage allows precise and continuous mapping of the sea floor. Side scan sonar delineates the edge of disposal deposits more accurately than bathymetric data.	
Limitations	The interpretation of side scan images requires some training and experience.	
Reference	US Army Engineer Waterways Experiment Station. 1988. Acoustic Tools and Techniques for Physical Monitoring of Aquatic Dredged Material Disposal Sites. EEDP-01-10. Waterways Experiment Station, U.S. Army Corps of Engineers, Vicksburg MS.	
Website	http://www.wes.army.mil/el/dots/pdfs/eedp01-10.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.1-15	
Method Title	Settlement Plates, DRP-2-03	
Purpose	To monitor changes in thickness of various layers of dredged and capping material in confined upland or aquatic disposal sites.	
Method Summary ISC	Telescoping settlement plates are used to measure changes in height of individual material layers at disposal site. The lower tier plate is placed on the foundation sediment before the dredged material is deposited. After dredged material disposal, a second tier settlement plate is slipped over the riser pipe of the lower tier and comes to rest on the surface of the dredged material. After placement of the cap, a third tier settlement plate is placed over the riser pipe of the second tier, and the plate rests on the surface of the cap. Readings are made to determine changes in individual layer thickness. Riser pipes and settling plate can be constructed from a variety of materials. For aquatic disposal sites, settlement plates can be designed and constructed to have a unit weight approximating that of water so that the plates do not sink through the soft dredged material or cause consolidation of the underlying material.	
Data Uses/Application	Monitoring sediment consolidation at dredged material disposal sites, consolidation of cap at ISC sites, and consolidation of sediment and/or cap at CAD sites.	
Advantages	Exact changes in thickness (*i.e.,* settlement) of various layers of deposited material can be directly measured. Settlement of the foundation sediments can be determined using a stationary benchmark relative to the lower tier riser pipe.	
Limitations	Divers must be used to place the plates and make the settlement readings. The riser pipes/settlement plates are vulnerable to accidental disturbance, removal, or damage by boating, fishing, or dredge disposal activities.	
Reference	US Army Engineer Waterways Experiment Station. 1989. Monitoring Dredged Material Consolidation and Settlement at Aquatic Disposal Sites, Environmental Effects of Dredging Technical Notes EEDP-01-5. Waterways Experiment Station, U.S. Army Corps of Engineers, Vicksburg MS.	
Website	http://www.wes.army.mil/el/dots/pdfs/eedp01-5.pdf	Last Accessed: 1/30/2003

2.2.2 Chemical and Physical Analysis

Section 2.2.2 contains methods for sample preparation and the chemical and physical analysis of sediment and soil. These methods characterize the chemical composition and physical properties of sediment/soil samples collected by methods described in Section 2.2.1. Samples are often analyzed for the presence of various inorganic and organic contaminants that may pose a threat to human or ecological health. Many of the methods described have been developed over time to optimize the detection, identification, and quantification of potential chemicals of concern. Several are performance-based and may be further modified to enhance the accuracy and precision of the method.

A variety of methods may exist for the analysis of a particular chemical parameter, all with varying levels of quantification or degrees of sensitivity. Less sensitive methods may be used as a screening tool during the initial site assessment to identify potential chemicals of concern. Follow-up analysis may include the use of very precise methods that provide unequivocal identification and trace level quantification of analytes. This variety also provides alternative methods useful in the analysis of many types of sediment or soil samples. Interferences from certain compounds in a sample may be avoided by the use of an alternative preparation or analytical method.

The physical properties of soil/sediment often influence the behavior of soil/sediment in the environment, and they may be helpful in further understanding the fate of contaminants associated with soil/sediment.

Many of the chemical and physical methods described in these fact sheets are routinely performed and fairly standardized. As a result, more than one source of information is often cited in each method description. Specifically, the following sources provided methods information for section 2.1.2:

- The USEPA's Office of Water
- The USEPA's Lake Michigan Mass Balance Study Methods Compendium, 1997v
- The USEPA's Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (SW846 Methods)
- NOAA's National Status and Trends Program, 1998
- Standard Methods for Examination of Water and Wastewater, 1999
- ASTM

Fact Sheet No.	2.2.2-1	
Method Title	Total Mercury in Sludge, Sediment, Soil, and Tissue by Acid Digestion and BrCl Oxidation, Appendix to Method 1631	
Purpose	These procedures may be used in conjunction with EPA Method 1631B for determination of mercury in sludge, sediment, soil, tissue, industrial samples, and certified reference materials.	
Method Summary	Digestion I—This procedure is preferred for matrices containing organic materials, such as sludge and plant and animal tissues, because the organic matter is completely destroyed. In this procedure, a 0.2 - 1.5 g sample is digested with HNO_3/H_2SO_4. The digestate is diluted with BrCl solution to destroy the remaining organic material. Digestion II—This procedure is preferred for geological materials because of rapid and complete dissolution of cinnabar (HgS), which is otherwise more slowly attacked by the BrCl in Digestion I. In this procedure, a 0.5 - 1.5 g sample is digested with aqua regia (HCl/HNO_3) to solubilize inorganic materials. The Hg concentration in the digestate is determined using EPA Method 1631B. These procedures, in conjunction with Method 1631B, allow determination of Hg at concentrations ranging from 1.0 to 5000 ng/g in solid and semi-solid matrices. The method detection limit for Hg has been determined to be in the range of 0.24 to 0.48 ng/g when no interferences are present. The minimum level of quantization (ML) has been established as 1.0 ng/g. These levels assume a sample size of 0.5 g. Method LMMB 050 (USEPA 1997b, EPA 905-R-97-012c) describes the automated digestion and analysis of total mercury in sediment samples using the Cold Vapor technique.	
Data Uses/Application	Mercury is one of the primary risk factors in many contaminated sediments and is one of the primary contaminants measured as part of a chemical assessment.	
Advantages	The dual amalgam trap system and fluorescence detector provide greater sensitivity and specificity in the presence of interferences, and this system must be used to overcome interferences, if present, and to achieve the sensitivity required, if necessary. For some site monitoring programs, total mercury is measured because it is a more rapid method compared to methyl mercury.	
Limitations	In cases where total mercury exceeds threshold values, samples may need to be analyzed for toxic methyl mercury to determine risk.	
Reference	USEPA. 2001d. Appendix to Method 1631: Total Mercury in Sludge, Sediment, Soil, and Tissue by Acid Digestion and BrCl Oxidation, EPA-821-R-01-013. Office of Water U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.brooksrand.com/FileLib/1631.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-2
Method Title	Trace Element Quantification Techniques
Purpose	To determine major and trace elements in sediment and biological tissue samples utilizing atomic absorption and neutron activation techniques.
Method Summary	Sediment samples are homogenized and freeze dried, and the dry aliquot is homogenized. Approximately 0.2 g of dried sediment is weighed and transferred to a Teflon™ bomb. Samples are digested by adding 3 mL HNO_3 and placing the bombs in a 130°C oven for approximately 12 hours. The bombs are removed, 2 mL of concentrated HF are added, and the bombs are returned to the oven for 12 hr. After cooling, 18 mL of 4% boric acid are added and the bombs are returned to the oven for another 12 hr. Solution volume is determined, and a 20-fold dilution is made for FAAS analysis of Al, Fe, Mn, Si, and Zn. For analysis of Hg, sediment samples are digested using a modified version of EPA method 245.5. Samples were analyzed using the following instrumentation: **Analyte** — **Method** Hg — Cold vapor atomic absorption (CVAA) Al, Fe, Mn, Zn — Flame atomic absorption (FAA) Ag, As, Cd, Cr, Cu, Ni, Pb, Se, Sn — Graphite furnace atomic absorption (GFAA) Al, Cr, Fe, Mn — Instrumental neutron activation analysis (INAA) ASTM Methods D1971, D3974, and D4698 and SW846 Method 3050B describe various digestion methods for determination of metals in sediments (ASTM, 2001a). Standard Method 3030K, ASTM Method D5258, and SW846 Method 3051 describe the microwave digestion method (APHA, 1999; ASTM, 2001a). Several methods describe the analysis of metals using various atomic absorption methods, such as Standard Method 3112 B for CVAA, 3111 for FAA, 3113B for GFAA and SW846 7000 series of methods.
Data Uses/Application	Chemical screening of trace metals in sediments against contaminant guidelines provides an indication that adverse effects may or may not be occurring.
Advantages	Tissue sample digestion in a Teflon™ bomb is a standard method for “clean” digestion for metals analysis. The instrumental suite employed in this method takes advantage of the know strengths of each instrument for trace analysis. For example, GFAA is much more sensitive than FAA, requiring only a small volume of sample for trace analysis, and CVAA is very sensitive for mercury.
Limitations	Processing of samples for trace levels required for risk assessments requires class-100 clean room, or other suitable environment.

Fact Sheet No.	2.2.2-2 (contd.)	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	http://ccma.nos.noaa.gov/publications/tm130.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-3
Method Title	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Purpose	To determine the concentration of 17 metals in sediment and biological tissue samples utilizing atomic absorption, inductively coupled plasma mass spectrometry (ICP-MS), and energy dispersive X-ray fluorescence (XRF).
Method Summary	Sediment samples are weighed and freeze-dried. The dried sample is ground in a ball mill. 0.5 g aliquots are used directly for XRF analysis or are further digested for AA or ICP-MS analysis. 200 mg of dried sediment is placed in a Teflon™ bomb, to which 1 mL of 4:1 $HNO_3/HClO_4$ is added. The bombs are heated in a 130°C oven for 4 hours. After cooling, 3 mL of concentrated HF are added to the bomb, and the bombs are heated again in a 130°C oven for 8 hours. After cooling, the digestates are diluted to approximately 20 mL with deionized water. Solution volumes are calculated, and the digestates are analyzed directly by GFAA or CVAA. For ICP-MS analysis, a 10-mL aliquot of the digestate is dried in a perchloric acid hood. The dried digestate is dissolved in 1 mL of 10% HNO_3 and dried again. The dried digestate is dissolved again in 1 mL of 10% HNO_3 and 9 mL of deionized water. **AnalyteMethod** Hg — Cold vapor/gold foil amalgam Cd, Se, Ag — Graphite furnace atomic absorption(GFAA) Ag, Al, Cr, Cd, Ni, Pb, Sb, Sn — ICP-MS Al, As, Cr, Cu, Fe, Mn, Ni, Pb, Si, Zn — XRF Standard Method 3030K, ASTM Method D5258, and SW846 Method 3051 describe the microwave digestion method (APHA, 1999; ASTM, 2001c). Selected methods for the analysis of metals include: Standard Methods 3112B for CVAA, 3113B for GFAA, and 3120B for ICP-MS; SW846 Method 6020 for ICP-MS and SW846 7000 series for atomic absorption (APHA, 1999).
Data Uses/Application	Chemical screening of trace metals in sediments against contaminant guidelines provides an indication that adverse effects may or may not be occurring.
Advantages	XRF analysis does not require digestion of the sample. Crustal elements such as Al, Cr, Fe, Ni, and Si, that can be difficult to dissolve from sediment, can be quantified by XRF. ICP-MS has the advantage of simultaneous analysis of many elements with detection limits much lower than the XRF and similar to those of GFAA. ICP-MS is particularly sensitive for Al, Cr, Ni, Ag, Cd, Sn, Sb, and Pb. CVAA is very sensitive and reliable for Hg analysis.
Limitations	Leakage at high pressure can cause loss of Hg from the sample during digestion. Analysis of GFAA requires the use of matrix modifiers and standardization of the instrument by method of addition to the sample matrix to provide accurate results. XRF and total HF digestion measure total metals, which may not reflect levels that are bioavailable.

Fact Sheet No.	2.2.2-3 (contd.)	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	http://ccma.nos.noaa.gov/publications/tm130.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-4	
Method Title	Determination of Acid Volatile Sulfide and Selected Simultaneously Extractable Metals in Sediment	
Purpose	For the determination of acid volatile sulfide (AVS) and for selected metals that are solubilized during the acidification step (simultaneously extracted metal, SEM).	
Method Summary	Sediment samples must be protected from exposure to oxygen during collection and storage. If possible, the head space in the sample container should be filled with oxygen-free nitrogen or argon. Appropriate storage conditions: frozen (preferred if sediments to be used for chemical analyses only) or refrigerated to 4 degrees Celsius (if sediments to be used for biological tests as well); glass containers if refrigerated, but plastic is acceptable if frozen. About 10 gm of sediment is acidified with hydrochloric acid at room temperature to convert the AVS to hydrogen sulfide. The H_2S is then purged from the sample and trapped in an aqueous solution, which varies depending upon the analytical method being used. Using the colorimetric method, the H_2S is trapped in sodium hydroxide. The sulfide reacts with N-N-dimethyl-p-phenylenediamine to form methylene blue, which is then measured colorimetrically at 670 nm. Using the gravimetric method, the sulfide is trapped in silver nitrate, forming a silver sulfide precipitate. The silver sulfide is isolated onto a 1.2 micron filter by filtration. The filter is dried and the amount of silver sulfate is weighed. Using the third method, the sulfide is trapped in an antioxidant buffer, and the sample is analyzed using an ion-selective sulfide electrode. After the determination of AVS, the acidified sediment sample is filtered through a 0.2µ membrane filter. The filtrate is analyzed for SEM (commonly, cadmium, copper, lead, silver, nickel, and zinc) by atomic absorption or inductive coupled plasma spectrometric methods. Both AVS and SEM are expressed on a µmole per gram dry sediment basis. The ratio of SEM to AVS is the sum of the concentrations of SEM metals divided by the acid volatile sulfide concentration.	
Data Uses/Application	Sulfide is important in controlling the bioavailability of metals in anoxic sediments. The amounts of SEM and AVS are important in predicting the bioavailability of metals.	
Advantages	The gravimetric procedure can be used with samples that have a moderate or high AVS concentration. The colorimetric method is capable of determining AVS concentrations over a range of 0.01-1000 µmoles/gram dry weight. The sample purging and trapping apparatus may consist of either Erlenmeyer flasks (less costly) or ground glass stoppered flasks (better sealing).	
Limitations	Sulfide ion is unstable in the presence of oxygen. Sulfide can be formed or lost due to biological activity during storage. Leakage of the Erlenmeyer flasks may cause low recovery of AVS.	
Reference	USEPA. 1991. Draft Analytical Method for Determination of Acid Volatile Sulfide in Sediment, EPA-821-R-91-100. Office of Water, U.S. Environmental Protection Agency, Washington, DC. Proper storage of samples will mitigate these limitations.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.2-5	
Method Title	Photovac GC Analysis for Soil, Water, and Air/Soil Gas, OSWER SOP# 2109	
Purpose	This method is designed as a field screening procedure for the tentative identification of various volatile organic compounds.	
Method Summary	Soil samples are collected in 40-mL VOA vials with Teflon™-lined silicone septum screw caps. A 5 gm aliquot of sample is weighed into a second, clean VOA vial. Reagent water is added to the sample to bring the volume in the vial to 20 mL. The vial is capped, shaken vigorously for one minute, and allowed to stand at room temperature for at least one hour for vapor phase equilibration. An aliquot of the soil head space is then removed from the vial and injected into the GC using a gas-tight syringe. The GC uses an ultraviolet light source and photoionization detector. Concentrations are reported as µg/kg. Typical MDLs for this method range from 1 ppb to 5 ppb. SOPs # 2108 and #2107 describe the operation of specific models of Photovac Gas Chromatographs.	
Data Uses/Application	Site assessment/characterization and health and safety surveys.	
Advantages	The data generated with this method allows for rapid evaluation of site conditions.	
Limitations	Pollutant identification is only tentative.	
Reference	USEPA. 1994b. SOP # 2109: Photovac GC Analysis for Soil, Water, and Air/Soil Gas. Compendium of Emergency Response Team Standard Operating Protocols. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison NJ.	
Website	http://www.ert.org/products/2109.PDF	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-6
Method Title	Extraction and Clean-Up of Sediments for Semi-volatile Organics Following the Internal Standard Method, LMMB 040
Purpose	To prepare the sediment samples for the measurement of organic contaminants, such as polychlorinated biphenyls, polynuclear aromatic hydrocarbons, and chlorinated pesticides.
Method Summary	Following Method LMMB 040 (USEPA 1997d: EPA 905-R-97-012c), 15 - 30 g of sediment are chemically dried with sodium sulfate, spiked with surrogate standards, and extracted with DCM using a 30°C sonication bath (sonicate for 60 minutes, let stand in bath overnight [24 hours] and sonicate again for 60 minutes). During a silica/alumina column clean-up, two separate fractions are collected. The first fraction, which is eluded with hexane, contains PCBs, HCB, 4, 4'-DDE, aldrin, and heptachlor. The second fraction, which is eluded with 10% diethyl ether in hexane, contains alpha- and gamma-BHC, chlordanes, 4,4'-DDT, 4,4'-DDD, and all PAHs. The two fractions are concentrated. The first fraction is treated with activated copper and frozen at -15°C until analysis. The second fraction is solvent exchanged into hexane and refrigerated until analysis. Following NS&T procedures, samples are stored frozen at approximately -15°C until extraction. A 10 - 30 gram aliquot of the homogenized sediment sample is chemically dried with sodium sulfate, spiked with surrogate standards, and extracted with dichloromethane (DCM) using a Soxhlet apparatus for 8 hours. The extract is concentrated, filtered if necessary, and solvent changed to hexane. The sample is cleaned-up using purified silica gel/alumina column chromatography before instrumental analysis. Activated copper in the column removes elemental sulfur that may be present. The sample is concentrated to 1 mL in hexane for analysis. Chemical surrogates are used to monitor extraction and cleanup efficiency. Several methods for sample extraction and clean-up are described in SW846 Methods 3500B and 3600C. ASTM Method D3976 describes the preparation of sediment samples for volatile, semi-volatile, and nonvolatile analyses (ASTM, 2001c).
Data Uses/Application	Polychlorinated biphenyls and high molecular weight PAHs are primary risk factors in many contaminated sediments and are measured as part of the chemical assessment of a site characterization and to assess remediation effectiveness.
Advantages	Both of the above listed methods, or variations based on performance, are sufficient for the analysis of PCB by GC-ECD or GC/MS (low resolution) or the analysis of PAH by GC/MS (low resolution).
Limitations	Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware. Matrix interferences result from co-extraction of compounds other than the analytes of interest, such as elemental sulfur and lipids.

Fact Sheet No.	2.2.2-6 (contd.)	
Reference	USEPA. 1997d. Method LMMB 040: Extraction and Clean-Up of Sediments for Semi-volatile Organics Following the Internal Standard Method in Lake Michigan Mass Balance Study Methods Compendium, Volume 2: Organic and Mercury Sample Analysis Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/lmmb/methods/sop-401.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-7	
Method Title	Quantitative Determination of Polynuclear Aromatic Hydrocarbons by Gas Chromatography/Mass Spectrometry (GC/MS) - Selected Ion Monitoring (SIM) Mode	
Purpose	To determine low concentrations of Polycyclic aromatic hydrocarbons (PAHs) and their alkylated homologues in extracts of water, sediments and biological tissues.	
Method Summary	Just prior to analysis, an aliquot of internal standard solution is added to the sample extract producing a final internal standard concentration of approximately 40 ng/mL. The analytical system includes a temperature programmable gas chromatography with a fused silica capillary column. Helium is used as the carrier gas, and the samples are handled by an auto sampler capable of making 1 - 4 µl injections. A five point calibration curve is established to demonstrate the linear range of the detector. The effluent from the GC capillary column is routed directly into the ion source of the mass spectrometer (MS). The MS is operated in the SIM mode using appropriate windows to include the quantization and confirmation masses for target PAHs. For all compounds detected at a concentration above the MDL, a confirmation ion is checked to confirm its presence. The response factors of the surrogate relative to each of the calibration standards are calculated, followed by the calculation of the sample extract concentration. The sample concentration for each compound is calculated by dividing the sample extract concentration by the sample amount.	
Data Uses/Application	PAH concentrations are primary risk factors associated with contaminated sediments. PAH data obtained from this analysis are used for site characterization and risk assessments.	
Advantages	GC/MS in the SIM mode provides unambiguous and sensitive detection for PAHs. The PAH quantization method is very rigorous because PAHs have very strong molecular ion peaks under the mass spectrometric conditions used. Alkylated PAH homolog data can be used for source identification. Also, the availability of labeled surrogates internal standards of many of the analytes makes very accurate determinations of analyte concentrations possible.	
Limitations	GC/MS in SIM mode cannot be used for simultaneous screening for other organic contaminants of similar polarity or volatility; cannot be used to identify tentatively identified compounds (TICs).	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	http://ccma.nos.noaa.govfpublications /tm30.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-8	
Method Title	Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection, LMMB 041	
Purpose	To quantify chlorinated hydrocarbons (*i.e.,* chlorinated pesticides and PCBs) in sample extracts.	
Method Summary	This method is based on high resolution, capillary gas chromatography using electron capture detection (GC/ECD). Extracts normally have a holding time of 40 days. The instrument's detector is calibrated before the sample in injected. Pesticide/PCB calibration is done also as part of the analytical run. If the response for any peak exceeds the highest calibration solution, the extract is diluted, a known amount of surrogate and TCMX solution added, and the sample reanalyzed for those analytes that exceeded the calibration range. Concentrations in the samples are calculated based on the internal standard method. Data is reported as ng/g dry weight. Other methods describing the analysis of PCBs and pesticides by GC/ECD are NS&T methods, ASTM Method E697, and SW846 Methods 8081A and 8082 (NOAA, 1998; ASTM, 2001d).	
Data Uses/Application	PCBs and persistent pesticides (particularly DDT and metabolites) are two of the primary risk factors of contaminated sediments. Data are used in site characterization and in risk analysis.	
Advantages	The ECD is very sensitive and allows for detection of the chlorinated hydrocarbons at trace concentrations (ppb).	
Limitations	The detector does not have a linear response over a wide concentration range and must be used by sufficiently trained personnel. Second column analysis must be performed to provide unequivocal compound identification. These methods do not measure the 12 World Health Organization PCB congeners, which may be desired data in some risk assessments. A separate analysis using a different GC column is required for peak confirmation.	
Reference	USEPA. 1997d. Method LMMB 041: Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection, in Lake Michigan Mass Balance Study Methods Compendium, Volume 2: Organic and Mercury Sample Analysis Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/lmmb/methods/sop-501.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-9	
Method Title	Screening for Polychlorinated Biphenyls by Immunoassay, SW846 Method 4020	
Purpose	This method is used to screen soils and non-aqueous waste liquids for the presence of polychlorinated biphenyls (PCBs).	
Method Summary	This method is used to determine when PCBs are present at concentrations above 5, 10 or 50 mg/kg. The method is most often performed using a sample extract. Determining the presence of PCBs above concentrations other than 5, 10 or 50 mg/kg is possible by dilution of the sample extract. The sample extract and an enzyme conugate reagent are added to immobilized antibody. The enzyme conjugate "competes" with PCB present in the sample for binding to immobile anti-PCB antibody. Test kits are commercially available for this method. Each commercially-available test kit will supply or specify the apparatus and materials necessary for successful completion of the test. The manufacturer's directions should be followed. Method 4020 provides an estimate for the concentration of PCBs by comparison with a standard.	
Data Uses/Application	Site characterization, screening.	
Advantages	Test kits are commercially available for this method.	
Limitations	This method does not provide information regarding congener or Aroclor concentrations. High levels of chemically-similar compounds may register a false positive. Method is intended for screening, not for quantitative analysis. In cases where the exact concentrations of PCBs are required, quantitative techniques should be used. If the proportions of PCB congeners in the calibration standard are not similar to the proportions present at the site, accuracy can be compromised.	
Reference	USEPA. 1996h. Screening of Polychlorinated Biphenyls by Immunoassay, SW846 Method 4020. Office of Solid Waste, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/epaoswer/hazwaste/test/pdfs/4020.pdf	Last Accessed: 2/13/03

Fact Sheet No.	2.2.2-10
Method Title	Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, EPA Method 1613
Purpose	This method is for determination of tetra- through octa-chlorinated dibenzo-p-dioxins (CDDs) and dibenzo furans (CDFs) in solids (not tissue).
Method Summary	This method is "performance-based". The labeled compounds are spiked into a sample containing 10 g (dry weight) of solids. Samples containing multiple phases are pressure filtered and any aqueous liquid is discarded. Coarse solids are ground or homogenized. Any non aqueous liquid from multi phase samples is combined with the solids and extracted in an extractor. The extract is concentrated for cleanup. After extraction, $_{37}Cl_4$-labeled 2,3,7,8-TCDD is added to each extract to measure the efficiency of the cleanup process. Sample cleanups may include back-extraction with acid and/or base, and gel permeation, alumina, silica gel, Florisil and activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of the 2,3,7,8-isomers or other specific isomers or congeners. After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, internal standards are added to each extract, and an aliquot of the extract is injected into the gas chromatography. The analytes are separated by the GC and detected by a high-resolution (≥10,000) mass spectrometer. **CDD/CDF — Minimum Level (ng/kg) — CDD/CDF — ML(ng/kg)** 2,3,7,8-TCDF 1 — 1,2,3,4,7,8-HxCDD 5 2,3,7,8-TCDD 1 — 1,2,3,6,7,8-HxCDD 5 1,2,3,7,8-PeCDF 5 — 1,2,3,7,8,9-HxCDD 5 2,3,4,7,8-PeCDF 5 — 1,2,3,4,6,7,8-HpCDF 5 1,2,3,7,8-PeCDD 5 — 1,2,3,4,7,8,9-HpCDF 5 1,2,3,4,7,8-HxCDF 5 — 1,2,3,4,6,7,8-HpCDD 5 1,2,3,6,7,8-HxCDF 5 — OCDF 10 1,2,3,7,8,9-HxCDF 5 — OCDD 10 2,3,4,6,7,8-HxCDF 5 This method is also described in SW846 Method 8290 and NS&T methods (NOAA 1998).
Data Uses/Application	The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.
Advantages	Method 1613 is able to meet detection limits required for human health and ecological risk assessments.
Limitations	The GC/MS portions of this method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons.
Reference	USEPA. 1994c. Method 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, EPA 821-B-94-005. Office of Water, U.S. Environmental Protection Agency, Washington, DC.
Website	http://www.epa.gov/waterscience/methods/1613.pdf — Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-11
Method Title	Toxic Polychlorinated Biphenyls by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry, EPA Method 1668
Purpose	This method is for determination of the toxic polychlorinated biphenyls (PCBs) in solids (not tissue).
Method Summary	This method is performance-based. The labeled compounds are spiked into a sample containing 10 g (dry weight) of solids. Samples containing multiple phases are pressure filtered and any aqueous liquid is discarded. Coarse solids are ground or homogenized. Any non-aqueous liquid from multi-phase samples is combined with the solids and extracted in an SDS extractor. The extract is concentrated for cleanup. After extraction, samples are cleaned up using back-extraction with sulfuric acid and/or base, and gel permeation, silica gel, Florisil and activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of specific isomers or congeners. After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, internal standards are added to each extract, and an aliquot of the extract is injected into the gas chromatography. The analytes are separated by the GC and detected by a high-resolution (≥10,000) mass spectrometer.

IUPAC	**EMDL (ng/kg)**	**EML (ng/kg)**	**Extract EML (pg/µL)**
77	0.5	2	1
123	4	10	5
126	10	4	5
118/167/156/157/169/180/170/189	6	20	10
114	60	200	100
105	40	100	50

EMD: = Estimated Method Detection Limit; EML = Estimated Minimum Level

Data Uses/Application	The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.	
Advantages	Method 1668 provides data for most, but not all, of the "dioxin-like" PCBs, including those with the highest TEFs, as determined by the World Health Organization. This method provides detection limits frequently required in risk assessments.	
Limitations	The GC/MS portions of this method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons. Method 1668 does not provide data for all of the "dioxin-like" PCBs, as does Method 1668A.	
Reference	USEPA. 1997e. Method 1668: Toxic Polychlorinated Biphenyls by Isotope Dilution HRGC/HRMS, EPA-821-R-97-001. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-12	
Method Title	Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, EPA Method 1668 Revision A	
Purpose	This method is for congener-specific determination of more than 150 chlorinated biphenyl (CB) congeners in solids (not tissue).	
Method Summary	This method is performance-based. The labeled compounds are spiked into a sample containing 10 g (dry weight) of solids. Samples containing multiple phases are pressure filtered and any aqueous liquid is discarded. Coarse solids are ground or homogenized. Any non-aqueous liquid from multi phase samples is combined with the solids and extracted in a Soxhlet/Dean-Stark extractor. The extract is concentrated for cleanup. After extraction, a labeled cleanup standard is spiked into the extract which is then cleaned up using back-extraction with sulfuric acid and/or base, and gel permeation, silica gel, or Florisil chromatography. Activated carbon and high-performance liquid chromatography (HPLC) can be used for further isolation of specific congener groups. After cleanup, the extract is concentrated to 20 µL. Immediately prior to injection, labeled injection internal standards are added to each extract and an aliquot of the extract is injected into the gas chromatography (GC). The analytes are separated by the GC and detected by a high-resolution (≥10,000) mass spectrometer. Without interferences, EMDLs and EMLs will be, respectively, 0.5 and 1.0 ng/kg for soil, tissue, and mixed-phase samples, and EMLs for extracts will be 0.5 pg/uL. EMD: = Estimated Method Detection Limit; EML = Estimated Minimum Level	
Data Uses/Application	This Method is for use in data gathering and monitoring associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.	
Advantages	Method 1668A provides congener data that can be used for source identification. Listed PCBs include the 12 World Health Organization “dioxin-like” PCBs. The HRMS method provides lower EMDLs compared to ECD or low resolution MS analyses and provides unequivocal congener identification.	
Limitations	The GC/MS portions of this method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons. Solvents, reagents, glassware, and other sample processing hardware may yield artifacts, elevated baselines, and/or lock mass suppression causing misinterpretation of chromatograms.	
Reference	USEPA. 1999c. Method 1668, Revision A: Chlorinated Biphenyl Cogeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, EPA-821-R-00-002. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/region08/water/wastewater/biohome/biosolidsdown/methods/1668a5.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-13	
Method Title	Butyltin in Sediments	
Purpose	The extraction and analysis of organotin compounds in soil and sediment.	
Method Summary	A 15 g aliquot of freeze-dried sediment sample is spiked with surrogate standards and shaker extracted four times with 0.2% tropolone in dichloromethane. The extract is concentrated by Kuderna-Danish technique and solvent exchanged to hexane. Organotin compounds are hexylated with hexylmagnesium bromide (Grignard reagent) by adding the reagent to the sample and heating the sample at 70°C for 30 minutes. The excessive reagent is removed with HCl and the organic phase of the sample removed. The remaining aqueous phase is extracted twice with pentane: CH_2Cl_2 (3/1, v/v). The combined hexylated extract is dried with sodium sulfate and concentrated. The sample is loaded onto a silica gel/alumina column and eluded with pentane. The cleaned sample is concentrated and analyzed by high resolution, capillary gas chromatography using flame photometric detection (GC/FPD). This method quantitatively determines Tetra butyltin (4BT), tributyltin (TBT), dibutyltin (DBT), and monobutyltin (MBT). Results are reported as ng Sn/g.	
Data Uses/Application	Butylin is a principal risk factor in many freshwater and marine harbor sediments.	
Advantages	FPD is a sensitive detector that is specific to tin. Hexylation of the organotin anions provides compounds amenable to the GC/FPD technique and provides reliable quantization of organotins at low concentrations (ng Sn/g).	
Limitations	Organotins are ubiquitous laboratory contaminants. Clean methods must be observed.	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	http://ccma.nos.noaa.govfpublications /tm130.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-14	
Method Title	Procedures for Sediment Total Organic Carbon (TOC) Determination	
Purpose	Determination of the TOC content in a sample by combustion.	
Method Summary	This method describes TOC determination by combusting preacidified samples at high temperature and measuring the volume of carbon dioxide gas produced. Samples are homogenized and 5 g wet weight subsamples dried for approximately 48 hours in a covered evaporation dish. The dried sediment sample is then ground with a porcelain pestle. Approximately 20 to 30 mg of the dried and ground sediment sample are placed in small beakers and acidified to remove sources of inorganic carbon. Samples are then dried again and then exposed to a pre-combusted stream of oxygen. The CO_2 evolved is measured by an infrared gas analyzer and the resulting gas peak is integrated. Integrator units are compared to a standard curve to convert to organic carbon. Alternatively, using the NS&T method, 0.1 to 0.5 ± 0.001 g of oven-dried, finely ground homogenized sediment is weighed into a combustion crucible (NOAA, 1998). Approximately 1.4 g each of copper and iron chip accelerators are added to the crucible. The crucible is placed and sealed within the oven combustion tube. Total carbon compounds in samples are decomposed by pyrolysis in the presence of oxygen, and the CO_2 that is formed is quantified by infrared detection. Total organic carbon (TOC) is determined by acidifying the sample with 10% HCl and drying the sample overnight at 50°C. Acidification converts carbonate carbon to carbon dioxide, which is purged from the acidified sample prior to analysis. Carbonate carbon, or total inorganic carbon (TIC), is determined by the difference between total carbon and total organic carbon.	
Data Uses/Application	TOC is used to normalize the concentration of nonionic organic contaminants in the development of equilibrium partitioning sediment guidelines (ESGs), and this is an important chemical parameter for sediment quality.	
Advantages	The EMAP method is one of several combustion methods for TOC determination.	
Limitations	The TOC determination is not a substitute for the determination of biological oxygen demand or chemical oxygen demand, should those parameters be needed.	
Reference	USEPA. 1995. Environmental Monitoring and Assessment Program Laboratory Methods Manual, Estuaries, Volume 1-Biological and Chemical Analyses, EPA/620/R-95/008. Environmental Monitoring and Assessment Program , U.S. Environmental Protection Agency, Washington DC.	
Website	http://www.epa.gov/emap/html/pubs/docs/groupdocs/estuary/field/lab_man.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-15	
Method Title	Determination of the Activity of Lead-210 in Sediments and Soils, LMMB 084	
Purpose	This procedure measures the activity of the Pb-210 granddaughter, Po-210.	
Method Summary	Sediment is collected with a gravity or box corer. The samples are extruded at known intervals and placed into preweighed bottles. The samples are dried in a 60°C oven, and the dry weight calculated. The samples are then ground to a fine powder and stored until used. 0.50 g of dried sediment is digested with a combination of HCl and hydrogen peroxide. During the digestion, the sample is heated on a hot plate to 90-95°C for a total of four hours. After sitting overnight, the sample is filtered through a Whatman No. 42 filter paper into a flask. The sample is heated until its volume is reduced to 5 mL. The pH of the sample is adjusted to 0.5 to 1.0. Ascorbic acid is added to prevent interference from ferric iron. A copper disk is added to the sample, which is heated overnight in a 95°C oven. The Po-210 concentration on the disk is determined by alpha spectrometry using silicon surface barrier detectors . A yield monitor, Po-208, is added to each sample to determine the exact activity of Po-210.	
Data Uses/Application	The activity of Pb-210 can be used to estimate dates of sediment deposition. Such dating can be used in establishing chronologies of sediment contamination, rates of sediment accumulation and rates of contaminant attenuation.	
Advantages	Straight forward and accurate analytical method.	
Limitations	Pb-210 has a half life of 22 years, limiting age dating using Pb-210 to approximately the last 100 years. If sediments have been disturbed (*e.g.*, by past dredging, scouring, bioturbation), accurate dating may not be possible. It should be noted that other analyses may be more accurate for dating sediment deposition which occurred more recently than the last 100 years. For example, analysis for Cesium-137 may be suitable for dating sediments deposited in the previous 50 years, while Beryllium-7 can date sediments deposited in the previous two years. Using more than one dating method can provide added assurance in date estimates.	
Reference	USEPA. 1997c. Method LMMB 084: Determination of the Activity of Lead-210 in Sediments and Soils. Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chemistry, and Biomonitoring Sample Analysis Techniques. EPA 905-R-97-012c. U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/glnpo/lmmb/methods/lead-210.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-16	
Method Title	Sediment Grain Size Analysis, NHEERL-AED SOP 1.01.005	
Purpose	To determine the percentages by weight of sand, silt, and clay in sediment samples	
Method Summary	15 - 20 gm of sediment is treated with 50 - 100 mL of 30% hydrogen peroxide for 12 hours to oxidize any organic matter present. The sample is then washed with distilled water to remove salts. 400 mL of sodium hexametaphosphate solution is used to disperse the particles in the sediment. The sample is shaken for 24 hours. The gravel/sand fraction is wet-sieved through a .063 mm sieve into an underlying evaporation dish to separate the sands (>.063 mm) from the silt and the clay (<.063mm). The material remaining on the sieve(gravel/sand) is washed into a pre-weighed beaker. This fraction is dried at 100 - 130°C for 24 hours and then weighed to the nearest 0.1 g. The silt and clay in the evaporation dish is then transferred to a graduated cylinder and 10 mL of dispersant from stock solution of sodium hexametaphosphate is added. The solution is stirred and stored for 12 hours. If flocculation occurs, the sample is treated with more dispersant and mixed. The silt and clay fractions are measured using the pipette method (Folk, 1974). The solution is stirred and aliquots are pipetted out at specified times and depths. Silt and clay fraction: After 20 seconds of stirring, the pipette is inserted to 20 cm and at the end of 20 seconds, 20 mL is removed. This fraction is placed into a beaker. Clay fraction: After 2 hours and 3 minutes, the pipette is inserted to a depth of 5 cm and 20 mL is withdrawn. This fraction is placed into a beaker. The removed fractions are dried in an oven at 100 - 130°C for at least 24 hours. Total dry weight = wt. sand + wt. silt + wt. clay. The weight of silt+clay is multiplied by 50 and the weight of one dispersant is subtracted to obtain the total weight of silt+clay. This process is repeated for the clay fraction. Total dry weight = wt. sand + wt. silt + wt. clay. The respective percentages of sand, silt and clay are derived by dividing the individual weights by the total weight and multiplying by 100. Grain size analysis is also described in ASTM Method D422, NOAA NS&T, and EMAP Lab methods (ASTM, 2001e; NOAA, 1998).	
Data Uses/Application	Grain size is an important characteristic of sediments that may be correlated with contaminant concentrations. Data may also be used in sediment transport methods.	
Advantages	This is one of several methods derived from Folk (1974) that has gained wide acceptance for grain size determination.	
Limitations	This method does not provide Φ classification data; these data are important in some transport models.	
Reference	USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296. Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.01.005.pdf	Last Accessed:
Fact Sheet No.	2.2.2-17	

Method Title	Procedures for Water Content Determination	
Purpose	Determine the percent water content of sediment samples.	
Method Summary	Sediments are placed in a 250 mL glass beaker and homogenized. Approximately 5-10 grams wet weight of sediment will be placed in a clean, tared 50 mL glass beaker and the weight are recorded. The sample is dried for 24 hours and then reweighed. Percent water content is then calculated. Alternatively, using the NS&T method, sediments are homogenized using a solvent rinsed spatula (NOAA, 1998). Approximately five grams of sample is placed in a pre-weighed scintillation vial (combusted for 4 hr at 400°C) and the weight recorded. The samples are dried for 24 hours in a drying oven set at 63-65°C. Samples are placed in a desiccator and allowed to cool to room temperature for at least 30 minutes. The samples are weighed. The samples are put back in the oven for at least 2 hr after which they are removed from the oven and allowed to cool for at least 30 min in a desiccator. The sample is reweighed. If the difference between the first and second weighting wis less than ± 0.02 g, the dry weight percent is calculated based on the last weighing. $\text{Percent dry weight} = \frac{[\text{Vial wt.} + \text{Dry sample wt.}] - [\text{Vial wt.}]}{[\text{Vial wt.} + \text{Wet sample wt.}] - [\text{Vial wt.}]} \times 100$ The percent water content is calculated as 100 - percent dry weight.	
Data Uses/Application	Dry weight measurements are used to calculate sediment analyte concentrations on a dry weight basis. In addition, water content is one of a suite of parameters typically used in settlement calculations for contaminated sediment capping studies. The EPA Region I functional guidelines use percent solids data as follows. If the percent solids content of a soil/sediment sample falls below 30%, all positive and non-detect results in that sample are qualified (J for positive results, R for non-detects). When the percent solids content of a soil/sediment sample falls below 10%, all results in that sample are rejected (R).	
Advantages	Dry weight measurements determined in this manner can be compared directly to NOAA NS&T database.	
Limitations	This method has not been widely accepted as the method for drying to constant weight at 105° C.	
Reference	USEPA. 1995. Environmental Monitoring and Assessment Program Laboratory Methods Manual, Estuaries, Volume 1-Biological and Chemical Analyses, EPA/620/R-95/008. Environmental Monitoring and Assessment Program , U.S. Environmental Protection Agency, Washington DC.	
Website	http://www.epa.gov/emap/html/pubs/docs/groupdocs/estuary/field/lab_man.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-18	
Method Title	Standard Test Method for Field Vane Shear Test in Cohesive Soil, ASTM Method D2573	
Purpose	To determine the shear strength of cohesive soils.	
Method Summary	A four-bladed vane is placed in the undisturbed soil and is rotated from the surface to determine the torsional force required to cause a cylindrical surface to be sheared by the vane. Friction of the vane rod and instrument must be accounted for, so as not to record friction as soil strength. Shear is calculated as torque multiplied by the inverse of a constant, depending on the dimensions and shape of the vane.	
Data Uses/Application	Field vane shear testing measures the *in situ* undrained shear strength of sediments underlying the surficial low- bearing capacity sediments. In-situ contaminated sediments to be capped are predominately fine-grained, and may have high water contents and low shear strengths. The shear strength of sediments will influence their resistance to localized bearing capacity or sliding failures, which may cause localized mixing of capping and contaminated materials.	
Advantages	There are cost and schedule advantages to performing this procedure in the field.	
Limitations	This test should not be performed in any soil such as sand or silt that will permit drainage during the test period or in soils where stones or shells are encountered by the vane.	
Reference	ASTM. 2001e. ASTM Book of Standards. Volume 04.08. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.2-19	
Method Title	Standard Test Method for Specific Gravity of Soil Solids by Water Pycnometer, ASTM Method D854	
Purpose	To determine the specific gravity of soil solids that pass the 4.75-mm sieve, by means of a water pycnometer..	
Method Summary	Two alternative methods exist for measuring specific gravity: procedure for moist specimens and procedure for oven-dry specimens. Guidelines exist that recommend the dry soil mass versus soil type and pycnometer size. After these guidelines have been consulted and the sample size selected, the pycnometer volume is calibrated. The pycnometer is then weighed. Following the procedure for moist samples, the water content of the sample is determined, and the sample is mixed with 100 mL of water to form a slurry. The slurry is transferred into the pycnometer. Following the procedure for oven-dry specimens, the sample is dried to a constant mass in a 100°C oven and then transferred to the pycnometer. Water is then added to the pycnometer to form a slurry, and the slurry is deaired using either heat, vacuum, or a combination of both. The pycnometer is filled with deaired water and placed into an insulated container, where it is left overnight to thermally equilibrate. The following day, the mass of the pycnometer, soil, and water are determined, and the temperature of the slurry is measured. The soil slurry is transferred to a tared pan and dried to a constant mass at 110°C. The specific gravity of soil solids at test temperature is calculated using the following equation: $G_t = \frac{M_s}{(M_{pw,t} - (M_{pws,t} - M_s))}$, where M_s = the mass of the oven dry soil solids (g) $M_{pw,t}$ = the mass of pycnometer and water at the test temperature (g) $M_{pws,t}$ = the mass of pycnometer, water, and soil solids at the test temperature (g) The specific gravity of soil solids at 20°C is calculated by multiplying the specific gravity at the test temperature by a temperature coefficient.	
Data Uses/Application	Specific gravity is used to calculate the phase relationship of soils, such as void ratio and degree of saturation, and soil solid density. In addition, specific gravity is one of a suite of parameters typically used in settlement calculations for contaminated sediment capping studies.	
Advantages	This is the standard, accepted method for measuring specific gravity in soil.	
Limitations	This analysis cannot be performed on soil solids that may be altered by this method or on highly organic soil solids.	
Reference	ASTM. 2001e. ASTM Book of Standards. Volume 04.08. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.2-20	
Method Title	Standard Test Method for Permeability of Granular Soils (Constant Head), ASTM Method D2434	
Purpose	To determine the coefficient of permeability by a constant-head method.	
Method Summary	A representative sample of soil is analyzed for particle size before the permeability test. Any particles larger than 19 mm are separated out by sieving and the percentage of oversize material is recorded. Of the sieved sample, a subsample of approximately twice the amount required to fill the parameter chamber is selected by the method of quartering. First, the cross-sectional area of the sample is calculated. A subsample of the soil is analyzed for water content. The air-dried soil sample is spread and compacted in successive layers into the parameter until the device is filled to the proper level. The unit weights, void ratio, and relative density of the test specimen are calculated. Air adhering to soil particles and present in the voids is removed with a vacuum pump or aspirator. The sample is then saturated with water, preferably native water. The inlet valve from the filter tank is opened and time, head, quantity of flow and water temperature are recorded when a stable head condition is attained. Test runs are repeated at heads increasing by 0.5 cm in order to accurately establish the region of laminar flow with velocity directly proportional to hydraulic gradient. The coefficient of permeability is calculated as follows: $k = QL/Ath$, where k = coefficient of permeability, Q = quantity of water discharged, L = distance between manometers, A = cross-sectional area of specimen, t = total time of discharge, and h = difference in head on manometers. The permeability is corrected to that for 20°C.	
Data Uses/Application	Permeability measurements are needed for engineering sediment capping alternatives.	
Advantages	This is the standard and accepted method for measuring permeability in soil.	
Limitations	This procedure is limited to disturbed granular soils containing not more than 10% soil passing the 75-µm sieve.	
Reference	ASTM. 2001e. ASTM Book of Standards. Volume 04.08. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.2-21	
Method Title	Standard Test Method for One-Dimensional Consolidation Properties of Soil, ASTM Method D2435	
Purpose	To determine the magnitude and rate of consolidation of soil.	
Method Summary	This test procedure is usually performed on undisturbed samples of fine grained soils naturally sedimented in water. The sample is trimmed and placed in the tared consolidation ring. The sample is then trimmed flush with the plane ends of the ring. The initial wet mass, height, volume, and water content of the sample are determined. The sample is loaded into the consolidometer. The standard loading schedule consists of a load increment ration (LIR) of one which is obtained by doubling the pressure on the soil to obtain values of approximately 12, 25, 50, 100, 200, *etc.* kPa. The standard unloading schedule is selected by halving the pressure on the soil. Before each pressure increment is applied, the height or change in height of the sample is recorded. Two alternative procedures exist for the analysis of soil consolidation. Test Method A is performed with constant load increment duration of 24 hours, or multiples thereof. Time-deformation readings are required on a minimum of two load increments. Test Method B requires time-deformation readings on all load increments. Successive load increments are applied after 100% primary consolidation is reached, or at constant time increments as described in Test Method A. The deformation results are plotted (void ratio or strain), and the plot is used to determine the value of the preconsolidation pressure.	
Data Uses/Application	The data from the consolidation test are used to estimate the magnitude and rate of both differential and total settlement of a structure or earthfill.	
Advantages	This is the standard and accepted method for measuring consolidation in soil.	
Limitations	The test results can be greatly affected by sample disturbance and greatly dependent on the competence of the personnel performing the test.	
Reference	ASTM. 2001e. ASTM Book of Standards. Volume 04.08. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.2-22	
Method Title	Standard Test Method for Classification of Soils for Engineering Purposes (Unified Soil Classification System), ASTM Method D2487	
Purpose	To classify mineral and organo-mineral soils based on laboratory determination of a variety of parameters.	
Method Summary	This classification system identifies three major soil divisions: coarse-grained soils, fine-grained soils, and highly organic soils. These three divisions are further subdivided into a total of 15 basic soil groups. The minimum amount of test sample required for this test method depends on which of the laboratory tests need to be performed. The laboratory tests include particle-size determination, liquid limit, and plasticity index. The percentage of fines found in the sample dictates which tests are required. The results of these tests are used to classify soils by group.	
Data Uses/Application	Soil classification is used to describe a soil and to aid in the evaluation of its significant properties for engineering use.	
Advantages	Provides visual classification of soils for engineering purposes.	
Limitations	This classification system is dependent on the competence of trained personnel.	
Reference	ASTM. 2001e. ASTM Book of Standards. Volume 04.08. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.2-23	
Method Title	Standard Test Method for Liquid Limit, Plastic Limit, and Plasticity Index of Soils, ASTM Method D4318	
Purpose	To determine the liquid limit, plastic limit, and plasticity index of soils.	
Method Summary	The liquid and plastic limits of soils (collectively referred to as Atterberg limits) distinguish the boundaries of the several consistency states of plastic soils. These analyses are performed only on the portion of soil that passes through the 425-µm (No. 40) sieve. Two alternative methods exist for determining the liquid limit: the multipoint test (the recommended method) and the one-point test. The liquid limit is determined by conducting trials in which a portion of the sample is spread in a brass cup, divided in two by a grooving tool, and allowed to flow together which the cup is repeatedly dropped in a standard mechanical device. In the multipoint test, three or more trials are conducted over a range of water contents. In the one-point test, two trials at one water content is conducted. The plastic limit is determined by alternately pressing and rolling a portion of plastic soil into a 3.2 mm diameter thread until its water content is reduced to a point at which the thread crumbles and can no longer be pressed together and recoiled. The water content of the soil at this point is reported as the plastic limit. The plasticity index is calculated as the difference between the liquid limit and the plastic limit.	
Data Uses/Application	Results from these analyses are used in the soil classification process to characterize the fine-grained fractions of soils.	
Advantages	This is the standard, accepted method for measuring the plasticity of soils.	
Limitations	The one-point test is not recommended for inexperienced analysts. The one-point test may not be valid for certain types of soil, such as organic or marine soils.	
Reference	ASTM. 2001e. ASTM Book of Standards. Volume 04.08. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.2-24	
Method Title	Field Portable X-Ray Fluorescence Spectrometry for the Determination of Elemental Concentrations in Soil and Sediment	
Purpose	Site characterization, screening	
Method Summary	Under this method, inorganic analytes of interest are identified and quantified directly by a field portable X-Ray fluorescence spectrometer. Radiation from one or more radioisotope sources or an electrically excited X-Ray tube is used to generate the characteristic X-Ray emissions in a sample. Each source emits a specific set of primary x-rays that excite a corresponding range of elements in a samples. When more than one source can excite the element of interest, the source is selected according to its excitation efficiency for the element of interest. For measurement, the sample is positioned in front of the probe window. Samples may be analyzed in one of two manners: *in situ* or intrusive. If *in situ* mode, the probe window is placed in direct contact with the soil or sediment. In the intrusive mode, the sample is collected, prepared, and placed in a sample cup, which is then placed on top of the probe window inside a protective cover for analysis. Most FPXRF instruments are menu-driven from software developed by the manufacturer. The measurement time for each source is user-selectable. Shorter source measurement times (30 seconds) are used for initial screening or hot spot delineation. Longer times (up to 300 seconds) are used to meet higher precision and accuracy requirements.	
Data Uses/Application	Twenty-six elements can be measured by this method. Field-based detection limits established for Sb, As, Ba, Cr, Co, Cu, Pb, Mn, Mo, Ni, Rb, Sr, Sn, Zn, and Zr. Field screening of Cu, Pb, and Zn at metal contaminated sites has been demonstrated by the Navy (NFESC, 2000). Method 6200 is intended for dry samples. The Navy has demonstrated good results on unprepared, wet sediment (NFESC, 2000).	
Advantages	Rapid on-site screening for selected elements allows rapid characterization of many metal-contaminated sites.	
Limitations	High levels of V and Fe will interfere with the quantitation of Cr and Co, respectively. A high ratio of Pb to As may result in no As being reported regardless of actual concentration. Trained operators must understand the limitations of the method. Confirmatory laboratory analysis required.	
Reference	USEPA. 2000c. Method 6200, Field Portable X-Ray Fluorescence Spectrometry for the Determination of Elemental Concentrations in Soil and Sediment. Office of Solid Waste, U.S. Environmental Protection Agency, Washington DC.	
Website	http://www.epa.gov/epaoswer/hazwaste/test/pdfs/6200.pdf	Last Accessed: 1/30/2003

Fact Sheet No.	2.2.2-25	
Method Title	Sediment Age Dating Using Cesium-137	
Purpose	To determine chronological timescales in sediments using the radioisotope Cesium-137	
Method Summary	Sediment is collected with a coring device, as warranted by the sampling location. The samples are extruded from the corer in 5-cm segments and collected in sampling jars. A 100g aliquot is used for ^{137}Ce analysis. The sediment is homogenized, weighed and an aliquot is freeze-dried prior to analysis to determine percent solids. Sediment for analysis can be either wet or dry and does not require any special storage conditions. Sediment for ^{137}Ce analysis is gamma counted on a Ge-diode detector. The sample is placed in front of or on top of the detector, depending on the configuration of the system. The sample is placed in exactly the same position as the standard and this position remains constant for all samples in the batch. The sample is generally counted for 24 hours, depending on the size and activity of the sample.	
Data Uses/Application	The activity of Cesium 137 can be used to estimate dates of sediment deposition. Such dating can be used to establish chronologies of sediment contamination, rates of sediment accumulation and rates of contaminant attenuation.	
Advantages	Cesium 137 has a relatively short residence time in natural waters.	
Limitations	Cesium 137 is a thermonuclear byproduct. Its presence in natural systems is directly related to thermonuclear activity and therefore its useful in detecting effects since the 1950's only (onset of aboveground nuclear weapons testing). Particle size distribution (PSD, or grain size analysis) should be conducted concurrently to support the assumption that uniform sediment processes were occurring during the time of interest.	
Reference	Battelle. 2001. Natural Recovery of Persistent Organics in Contaminated Sediments at the Sangamo-Weston/Twelvemile Creek/Lake Hartwell Superfund Site. USEPA National Risk Management Research Laboratory Cincinnati, OH.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.2-26	
Method Title	Beryllium-7 as a Tracer of Short Term Sediment Deposition	
Purpose	To aid in the estimation of particle resuspension and deposition rates in sediments.	
Method Summary	Sediments are collected in the field with the appropriate sampler (e.g. core, grab, or sediment trap). Sediments are returned to the lab for analysis within 48 hours of collection. In the lab, the samples are dried to constant weight, ground, and homogenized using a mortar and pestle. The dried sediments are then compressed into pellets, weighed, and measured. The activity of ^{7}Be is determined on either a lithium-drifted or intrinsic germanium detector coupled to a multichannel autoanalyzer. The sample prep and analysis should take place in a shielded clean room. Prior to sample analysis the detectors are calibrated and a calibration curve is generated based on the sample size (in height). Measured counts per minute (cpm) are divided by the detector efficiency yielding disintegrations per minute (dpm). The activity is converted to picocuries per gram of dry weight and corrected for the detector efficiency.	
Data Uses/Application	^{7}Be is a naturally occurring, atomospherically derived radioisotope that can help provide actual rates of short-term deposition and resuspension of sediments. ^{7}Be can be used to elucidate types and rates of processes that directly affect the cycling of certain contaminants in the sediments, such as PCBs.	
Advantages	Because of it's relatively short half-life, ^{7}Be allows definition of short-term deposition and resuspension rates.	
Limitations	^{7}Be tracer analysis is useful in describing processes (short-term deposition and resuspension rates), and not particle type and composition.	
Reference	Fitzgerald, S.A., J Val Klump, PW Swarzenski, RA Mackenzie, and KD Richards. 2001. Beryllium-7 as a Tracer of Short-Term Sediment Deposition and Resuspension in the Fox River, Wisconsin. *Environmental Science and Technology* 35/ 300-305.	
Website	N/A	Last Accessed:

2.2.3 Biological Analysis Methods

Section 2.2.3 provides a compendium of sediment-related biological analyses. Table 2.2-1 lists numerous acute freshwater, chronic freshwater, acute marine, chronic marine and bioaccumulation tests that can be used to assess the effects that sediments may have on endemic organisms. Attention was paid to include ***reference all standard test species, different periods, endpoints. After performing physical analyses, the Superfund managers may select the test that best suits the physical, chemical and biological parameters at their site. These tests are performed to determine the lethal (acute) and sub-lethal (chronic) effects of the sediment on resident organisms. These results are then compared with chemistry data to identify and compare effects data with contaminant exposure data to determine the sediment's risk to ecological or human health.

The advantages and limitations associated with each test are provided in their respective fact sheet. However, there are general limitations and interferences associated with all active/chronic/freshwater/marine solid-phase toxicity tests. These potential interferences are identified by some of the source documents (ASTM Method E1706 (ASTM, 2001b), USEPA, 2000d). They are listed below:

- Sediment collection handling and storage may alter bioavailability;
- Natural geochemical characteristics of sediment may affect the response of test organism;
- Indigenous animals may be present in field-collected sediments;
- Route of exposure may be uncertain and data generated in sediment toxicity tests may be difficulty to interpret if factors controlling the bioavailability of contaminants in sediments are unknown;
- Tests applied to field samples may not discriminate effects of individual chemicals;
- Few comparisons have been made of methods or species;
- Only a few chronic methods for measuring sublethal effects have been developed or extensively evaluated; and
- Laboratory tests have inherent limitations in predicting ecological effects.

The toxicity test fact sheets provide methods described in USEPA guidance documents, Dredging Manuals, and ASTM reports from the following agencies and offices:

- The USEPA's Office of Water
- The USEPA's Office of Research and Development
- The USEPA and USACOE Dredging Teams
- The Puget Sound Water Quality Action Team
- The Puget Sound Dredged Disposal Analysis Program
- The USEPA's EMAP Program
- ASTM
- The USEPA's Environmental Research Laboratory-Narragansett,
- The USEPA's Great Lakes Program Office

Table 2.2.3-1. A Summary of Test Types and Toxicological Endpoints for Solid-Phase Toxicity

Test Type	Test Organism	Scientific Name	Endpoints	Test Specifics	Comments
Acute Freshwater 2.2.3-1 2.2.3-2 2.2.3-3	Amphipod Crustacean	*Ceriodaphnia dubia*	Survival	Static, Flow through and *in situ*, 48 hours	Commonly used for bioassays
Acute Freshwater 2.2.3-4	Crustacean other than amphipods	*Hyallela azteca*	Survival and growth	10 days	Short generation time, contact with sediment
Acute Freshwater 2.2.3-4	Insect Larvae	*Chironomus tentans*	Survival and growth	10 days	Short generation time, contact with sediment
Chronic Freshwater 2.2.3-5	Amphipod Crustacean	*Hyallela azteca*	Survival growth and reproduction growth	42 days	Short generation time, contact with sediment
Chronic Freshwater 2.2.3-5	Insect Larvae	*Chironomus tentans*	Survival, growth, reproduction, emergence of adults, egg number, and hatching success	Up to 60 days	Short generation time, contact with sediment
Acute Marine 2.2.3-7	Bivalve	*Mytilus edulis*	Larval Survival	48 hours	May need to substitute if sediments contain greater than 60% fines. Recommended at dredged material sites
Acute Marine 2.2.3-7	Bivalve	*Crassostrea gigas*	Larval survival, abnormal shell development	48 hours	May need to substitute if sediments contain greater than 60% fines. Recommended at dredged material sites
Acute Marine 2.2.3-8	Echinoderm	*Strongylocentrotus purpuratus*	Embryonic survival	48-96 hours	Recommended at dredged material sites
Acute Marine 2.2.3-8	Echinoderm	*Strongylocentrotus droebachiensis*	Acute/Embryonic survival	48-96 hours	Recommended at dredged material sites
Acute Marine 2.2.3-8	Echinoderm	*Dendraster excentricus*	Embryonic survival	48-96 hours	Recommended at dredged material sites
Acute Estuarine/Marine 2.2.3-9	Crustaceans other than amphipods	*Mysidopsis bahia*	Survival	96 hours	Wide tolerance to grain size, salinity and temperature. Epibenthos. Filter-feeder, deposit-feeder
Acute Estuarine/ Marine 2.2.3-9	Crustaceans other than amphipods	*Penaeus sp.*	Survival	96 Hours	Deposit-feeder, burrower

Table 2.2.3-1. (contd.)

Test Type	Test Organism	Scientific Name	Endpoints	Test Specifics	Comments
Acute Marine 2.2.3-10	Amphipod Crustacean	*Ampelisca abdita*	Survival	10 days	Wide tolerance to grain size, salinity and temperature. May be used if test sediment contains greater than 60% fines. Tube dweller, deposit-feeder, burrower. Recommended at dredged material sites
Acute Marine/Estuarine 2.2.3-10	Amphipod Crustacean	*Eohaustorius estuarius*	Survival	10 days	Wide tolerance to grain size, salinity and temperature. May be considered for use over grain size distributions ranging from 100% sand to 0.6% sand, as long as the clay fraction <30%; Free burrowing, deposit-feeder. Recommended at dredged material sites
Acute Marine 2.2.3-10	Amphipod crustacean	*Rhepoxynius abronius*	Survival	10 days	Preferred species for coarser-grained sediments (*i.e.,* fines <60%) Free burrowing, deposit-feeder. Recommended at dredged material sites
Acute Marine 2.2.3-11	Polychaete	*Neanthes arenaceodentata*	Survival	10 days	Size class must be uniform for biomass estimates. Tube dweller. Deposit-feeder, burrower. Recommended at dredged material sites Identified for bioaccumulation studies based on feeding type, biomass, salinity tolerance, pollution tolerance, culture potential, bioaccumulation toxicity information, commercial availability, and historic use in other programs.

Table 2.2.3-1. (contd.)

Test Type	Test Organism	Scientific Name	Endpoints	Test Specifics	Comments
Acute Marine 2.2.3-11	Polychaete	*Neanthes virens*	Survival	10 Days	Must be held under flow-through conditions. Deposit-feeder, burrower. Identified for bioaccumulation studies based on feeding type, biomass, salinity tolerance, pollution tolerance, culture potential, bioaccumulation toxicity information, commercial availability, and historic use in other programs.
Chronic Marine 2.2.3-12	Amphipod crustacean	*Leptocheirus plumulosus*	Survival, Growth, and Reproduction	28 days	Deposit-feeder, burrower
Chronic Marine 2.2.3-13	Polychaete	*Neanthes arenaceodentata*	Survival	28 days	same as above
Bioaccumulation Freshwater 2.2.3-17	Oligochaete	*Lumbrculus variegatus*	Bioaccumulation	28 days	Easy to culture and handle, tolerant of a wide range of sediment characteristics, and it is adaptable to long-term test exposures.
Bioaccumulation[1] Marine 2.2.3-17	Bivalve	*Macoma balthica*	Bioaccumulation	28 days	Identified based on feeding type, biomass, salinity tolerance, pollution tolerance, culture potential, bioaccumulation toxicity information, commercial availability, and historic use in other programs.
Bioaccumulation [1] Marine 2.2.3-17	Bivalve	*Macoma nasuta*	Bioaccumulation	28 days	Identified based on feeding type, biomass, salinity tolerance, pollution tolerance, culture potential, bioaccumulation toxicity information, commercial availability, and historic use in other programs.
Bioaccumulation [1] Marine 2.2.3-17	Bivalve	*Yoldia limatula*	Bioaccumulation	28 days	Identified based on feeding type, biomass, salinity tolerance, pollution tolerance, culture potential, bioaccumulation toxicity information, commercial availability, and historic use in other programs.

Table 2.2.3-1. (contd.)

Test Type	Test Organism	Scientific Name	Endpoints	Test Specifics	Comments
Bioaccumulation[1] Marine 2.2.3-17	Polychaete	*Neanthes virens*	Bioaccumulation	28 days	Must be held under flow-through conditions. Deposit-feeder, burrower. Identified for bioaccumulation studies based on feeding type, biomass, salinity tolerance, pollution tolerance, culture potential, bioaccumulation toxicity information, commercial availability, and historic use in other programs.
Bioaccumulation[1] Marine 2.2.3-17	Polychaete	*Nereis diversicolor*	Bioaccumulation	28 days	Identified for bioaccumulation studies based on feeding type, biomass, salinity tolerance, pollution tolerance, culture potential, bioaccumulation toxicity information, commercial availability, and historic use in other programs.

[1] For bioaccumulation tests, it is recommended that a deposit-feeding bivalve mollusk and a burrowing polychaete are used.

Fact Sheet No.	2.2.3-1	
Method Title	Acute Freshwater Crustacean Sediment Bioassay: Flow-through	
Purpose	This test is used to determine the effects of a 48-hour exposure of the freshwater crustacean, *Ceriodaphnia dubia*, to sediments in a laboratory flow-through system.	
Method Summary	200-mL aliquots of sediment (by weight) are placed in 1-L glass beakers, and 800 mL of site water is added slowly. *Ceriodaphnia* neonates are then placed in sediment exposure chamber (SEC) units and the units are placed in one of the test beakers containing sediment. The inlet port of the SEC unit connects to a reservoir (20 L) of site water. The flow-through rate is controlled by a metering pump calibrated to match the retention time of water flowing through the chambers in the field. Survival of *Ceriodaphnia* neonates is determined at the end of the 48-hour test.	
Data Uses/Application	The flow-through test allows investigators to better assess sediment contamination by simulating field conditions with water flow and controlling parameters such as pH and dissolved oxygen to examine for direct effects from contaminants.	
Advantages	*Ceriodaphnia dubia* have been used widely as a test species. It has shown to be a sensitive and useful test species for assessments of sediment toxicity.	
Limitations	Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	Sasson-Brickson, G. and G.A. Burton, Jr. 1991. *In Situ* and Laboratory Sediment Toxicity Testing with Ceriodaphnia Dubia. *Environmental Toxicology and Chemistry.* Vol 10. P 201-207.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.3-2	
Method Title	Acute Freshwater Crustacean Sediment Bioassay: *In Situ* Exposures	
Purpose	This test is used to determine the effects of a 48-hour exposure of the freshwater crustacean, *Ceriodaphnia dubia*, to sediments *in situ*.	
Method Summary	*Ceriodaphnia dubia* are transported to the test location and placed in one of five sediment exposure chambers (SEC). These acrylic chambers are tied together and placed onto the sediment surface. The inlet port of the SEC is directed upstream, so that it receives water that flows into the chamber, circulates and then exits via the outlet port, which is directed downstream. The SEC units are collected after 48-hours, placed in a polyethylene bucket containing site water, covered, and transported back to the laboratory, where surviving organisms were enumerated within 1.5 hours. Test-site water toxicity is differentiated from sediment toxicity by placing SEC units *in situ* with a plastic barrier between the unit base and the sediment surface. Water samples are also collected simultaneously in high-density polyethylene bottles and returned to the laboratory for toxicity testing.	
Data Uses/Application	*In situ* sediment exposures prove to be sensitive indicators of both degraded and nondegraded stream conditions. These types of experiments provide a way to compare and validate laboratory results to determine the accuracy of these laboratory experiments.	
Advantages	*Ceriodaphnia dubia* have been used widely as a test species. It has shown to be a sensitive and useful test species for assessments of sediment toxicity.	
Limitations	It is more difficult in the field to test effects exclusively from contaminants, since the investigators are unable to control all other parameters such as temperature, pH, and dissolved oxygen levels. Although the test is more representative of actual conditions, it is less definitive in correlating contaminant presence and ecological effects. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	Sasson-Brickson, G. and G.A. Burton, Jr. 1991. *In Situ* and Laboratory Sediment Toxicity Testing with Ceriodaphnia Dubia. *Environmental Toxicology and Chemistry.* Vol 10. P 201-207.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.3-3	
Method Title	Acute Freshwater Crustacean Sediment Bioassay: Static Laboratory Exposures	
Purpose	This test is used to determine the effects of a 48-hour exposure of the freshwater crustacean, *Ceriodaphnia dubia*, to sediments in a static laboratory system.	
Method Summary	To assess the partitioning of sediment-bound toxicants, the sediment is tested as solid phase, interstitial phase and elutriate phase. For solid-phase tests, sediments are homogenized in the laboratory with a hand paddle for approximately 5 minutes. The solid-phase test is prepared by placing 30 mL of wet sediment (by weight) into test beakers, then slowly adding 120 mL of reconstituted hard water with a syringe. Test beaker contents are allowed to settle for one hour prior to adding the test organisms. For interstitial phase tests, 400 to 450 mL of wet sediment is placed into a 500 mL polycarbonate bottle. The samples are centrifuged at 9000 rpm for 15 minutes. The resultant interstitial water is immediately siphoned off and placed in the test beakers. For elutriate phase tests, sediment and water are mixed in a 1:4 ratio by volume. Sediment is placed in a 500-mL polycarbonate bottle; 300 mL of reconstituted water is added to the bottle. The bottles are shaken on a Eberback shaker table for 30 minutes. After the mixture has settled, the liquid portion is siphoned off and centrifuged at 9000 rpm for 15 minutes. The supernatant water is siphoned off the top and added to the test beakers. *Ceriodaphnia* neonates are then exposed to test media (*i.e.,* whole sediment, interstitial water or elutriates) in 250-mL glass beakers containing 150-mL of the test solution. Beakers are maintained at 25 +/- 1° C. Survival numbers are recorded at 24 and 48 hours. Tests were considered valid when control mortality was < 10%.	
Data Uses/Application	This test method may be useful in assessing sediment contamination, registration of pesticides, assessments of new and existing chemicals, Superfund site assessment, and assessment and cleanup of hazardous waste treatment, storage, and disposal facilities.	
Advantages	*Ceriodaphnia dubia* have been used widely as a test species. It has shown to be a sensitive and useful test species for assessments of sediment toxicity.	
Limitations	Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	Sasson-Brickson, G. and G.A. Burton, Jr. 1991. *In Situ* and Laboratory Sediment Toxicity Testing with Ceriodaphnia Dubia. *Environmental Toxicology and Chemistry.* Vol 10. P 201-207.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.3-4
Method Title	Acute Freshwater Amphipod and Freshwater Insect Larvae Sediment Bioassay, EPA Method 100.1
Purpose	This test measures the survival and growth of the estuarine amphipod crustacean, *Hyallela azteca*, and the freshwater midge, *Chironomus tentans* after exposure to sediments for 10 days in the laboratory.
Method Summary	*Hyalella azteca* and/or *Chironomous tentans* are exposed to sediments for 10-days in a 300 mL test chamber containing 100 mL of sediment and 175 mL of overlying water that is renewed. Test temperature is 23 °+/- 1 °C. There are 10 organisms per chamber. The endpoints are survival and growth. Minimum mean control survival must be 70% and measurable growth of test organisms. Test organisms are fed daily. ASTM E1706-00 describes similar methods for conducting whole sediment toxicity tests with *Cladocerans*, mayflies, *Chironomus riporius, Diporeia spp* and Tubifex (ASTM Method E1706, ASTM 2001b). Environment Canada also describes a similar method using the test species *Hyalla azteca* in EPS/ 1/RM/33 and *Chironomus riparies* in EPS/1/RM/32.
Data Uses/Application	Sediments tests can be used to determine the relationship between toxic effects and bioavailability, investigate interactions among chemicals, compare the sensitivities of different organisms, determine spatial and temporal distribution of contamination, evaluate hazards of dredged material, measure toxicity as part of product licensing or safety testing, rank areas for clean up and estimate the effectiveness of remediation or management practices.
Advantages	Amphipods and midges are commonly used as appropriate test species to determine acute toxicity because they are relatively sensitive to contaminants associated with sediments, they have a short generation time, they have lots of contact with the sediment (*i.e.,* they are both deposit-feeders and burrowers), they are relatively easy to culture in the laboratory and they tolerate varying physico-chemical characteristics of sediment. Both the amphipod and midges are considered benchmark organisms by the USEPA and USACE Inland Testing Manual Standards (1998) and testing data comprise a substantial database. The organisms represent the sensitive range in a variety of ecosystems. These organisms provide comparative data on the relative sensitivity of local test species. The amphipod, *Hyallela azteca,* can also be used for estuarine toxicity studies; they are tolerant to salinities up to 25ppt.
Limitations	Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.

Fact Sheet No.	2.2.3-4 (contd.)	
Reference	USEPA. 2000d. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates. EPA/600/R-99/064. Office of Science and Technology, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/waterscience/cs/freshmanual.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.2.3-5	
Method Title	Chronic Freshwater Amphipod Sediment Bioassay, EPA Method 100.4	
Purpose	This test measures the survival, growth and reproduction of the freshwater crustacean, *Hyalella azteca, after* exposure to sediments for 42 days in the laboratory.	
Method Summary	The freshwater invertebrate, *Hyalella azteca*, is exposed to sediments for 42-days in a 300 mL container containing 100 mL of sediment and 175 mL of overlying water. Test temperature is 23 +/- 1°C. 100 mL of overlying water will be renewed every 12 hours. The organisms are fed daily. The endpoints are 28-day survival and growth, 35-day survival and reproduction; and 42-day survival, growth and reproduction. Survival is measured by counting the number of alive vs. dead amphipods at 28, 35 and 42 days. Reproduction is measured by exposing amphipods up until a few days before the release of the first brood. The amphipods are then sieved from the sediment and held in water to determine the number of young produced. Length and weight are measured to provide data for the growth endpoint.	
Data Uses/Application	This is a laboratory method for determining the chronic toxicity of contaminants associated with sediments collected from freshwater environments.	
Advantages	The amphipod, *Hyallela azteca*, is considered benchmark organism by the USEPA and USACE Inland Testing Manual Standards (1998) and testing data comprise a substantial database. The amphipod sensitivity corresponds to the sensitive range in a variety of ecosystems. This organism provide comparative data on the relative sensitivity of local test species.	
Limitations	The methodology recommended for measuring reproduction may not be accurate; the amphipods may recover from effects of sediment exposure during the holding period in clean water. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	USEPA. 2000d. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates, EPA/600/R-99/064. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/waterscience/cs/freshmanual.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.2.3-6	
Method Title	Life-Cycle Freshwater Midge Sediment Bioassay, EPA Method 100.5	
Purpose	This test measures the survival, growth and reproduction characteristics of the freshwater midge, *Chironomus tentans,* after exposure to sediments for 40 or 50 days in the laboratory.	
Method Summary	*Chironomus tentans*, a freshwater invertebrate, is exposed to sediments for up to 60 days in a 300 mL beaker containing 100 mL of sediment and 175 mL of overlying water. Test temperature is 23 +/- 1 °C. The endpoints are 20-day and end of test (50-60 days) survival, 20-day growth, and reproduction is monitored daily after day 23, examining endpoints such as emergence of adults, egg number and hatchling success. The end of the life-cycle test depends upon the sediments being evaluated. In clean sediments, the test typically requires 40 to 50 d from initial setup to completion. However, test duration will increase in the presence of environmental stressors which act to reduce growth or delay emergence. Where a strong gradient of sediment contamination exists, emergence patterns between treatments will likely become asynchronous, in which case each treatment needs to be ended separately.	
Data Uses/Application	This is a laboratory method for determining the chronic toxicity of contaminants associated with sediments collected from freshwater environments.	
Advantages	*C. tentans* is a good candidate for long-term toxicity testing because it has a short life cycle and a variety of developmental (growth, survivorship) and reproductivity (fecundity) endpoints can be monitored. The midge, *C. tentans*, is considered a benchmark species by the USEPA and USACE Inland Testing Manual Standards (1998) and midge testing data form a substantial database. Midge sensitivity is in the range of a many ecosystems, and midges provide comparative data on the relative sensitivity of local test species. They are burrowers and deposit-feeders.	
Limitations	Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	USEPA. 2000d. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, EPA/600/R-99/064. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/waterscience/cs/freshmanual.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.2.3-7	
Method Title	Acute Larval Bivalve Sediment Bioassay	
Purpose	This test measures the survival of the marine bivalve larvae, *Crassostrea gigas*, and *Mytilus edulis*, after exposure to sediments for 48 hours in the laboratory.	
Method Summary	Adult bivalves, conditioned as necessary in the laboratory, are induced to spawn with selected thermal and biological (*i.e.*, sperm) stimulation. Selected densities of the resulting embryos are exposed to the test or reference area sediments for 48 hours, during which the embryos normally will develop into prodissoconch I larvae. Exposure time should not exceed 60 hours for an acceptable test. Toxicity test endpoints are based on abnormal shell development and larval death.	
Data Uses/Application	This toxicity test can be used to assess the toxicity of marine sediments, especially dredged material. It may be used alone as a screening tool or in combination with sediment chemistry and *in situ* biological indices, and in laboratory experiments addressing a variety of sediment and water quality manipulations. These sediment bivalve bioassays are performed as a part of the Dredged Material Evaluation and Disposal Procedures in the Puget Sound (Puget Sound Dredged Disposal Analysis (PSDDA) Program, 2000).	
Advantages	Refer to table 2.2.3-1 to compare alternative methods for acute sediment bioassays.	
Limitations	Data from tests with longer exposures (> 48 hours) may not be comparable to those tests conducted using the standard 48-hour exposure. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	PSWQAT. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound, Puget Sound Protocols and Guidelines. Puget Sound Water Quality Action Team, Olympia, WA.	
Website	http://www.psat.wa.gov/Publications/protocols/protocol_pdfs/field.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.2.3-8	
Method Title	Acute Echinoderm Sediment Bioassay	
Purpose	This test measures the survival of the marine echinoderms, *Dendraster excentricus, Strongylocentrotus purpuratus* and *S. Droebachiensis,* after exposure to sediments for 48-96 hours in the laboratory.	
Method Summary	Adult Echinoderms, *Dendraster excentricus, Strongylocentrotus purpuratus* and *S. droebachiensis*, are induced to spawn with chemical stimulation. The resulting embryos are exposed to test sediment for 48 to 96 hours during which the embryos will develop into the four-armed pluteus stage. The toxicity test endpoint is based on failure to develop normal pluteus larvae. These sediment echinoderm bioassays are performed as a part of the Dredged Material Evaluation and Disposal Procedures in the Puget Sound. (USACE/WDNR/WDEC, 2000)	
Data Uses/Application	This toxicity test can be used to assess the toxicity of marine sediments, particularly dredged material. It may be used alone as a screening tool or in combination with sediment chemistry and *in situ* biological indices, and in laboratory experiments addressing a variety of sediment and water quality manipulations.	
Advantages	Refer to table 2.2.3-1 to compare alternative methods for acute sediment bioassays.	
Limitations	The three species may show different levels of sensitivity; therefore, the results for corresponding endpoints may not be comparable between the three species. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	PSWQAT. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound, Puget Sound Protocols and Guidelines. Puget Sound Water Quality Action Team, Olympia, WA.	
Website	http://www.psat.wa.gov/Publications/protocols/protocol_pdfs/field.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.2.3-9	
Method Title	Acute Marine Crustacean Sediment Bioassay	
Purpose	This test measures survival of the marine mysid shrimp, *Mysidopsis bahia* and the marine penaeid shrimp, *Penaeus* sp., after a 96-hour exposure to sediments in the laboratory.	
Method Summary	The mysid shrimp, *Mysidopsis bahia*, and Penaeid shrimp, *Penaeus* sp., are placed in 1-L glass chambers containing 175 mL sediment and about 800 mL of overlying water for 96-hours. Test temperature 20°C and the recommended overlying water salinity is 20 ppt. The test chambers will be lightly aerated, but water will not be renewed. Test species will be fed once daily. The endpoints in the toxicity test are survival. Performance criteria established for this test include the average survival of organisms in negative control treatment must be ≥ 90%. A miniaturized method of this test also exists (Ho, 2000) and will be incorporated into EPA's TIE guidance document.	
Data Uses/Application	This test method may be useful in assessing sediment contamination, registration of pesticides, assessments of new and existing chemicals, Superfund site assessment, and assessment and cleanup of hazardous waste treatment, storage, and disposal facilities.	
Advantages	Mysid shrimp are filter- and deposit-feeders commonly found in marine sediments; therefore, exposure to contaminated sediments is likely. Penaeid shrimp are deposit-feeders and burrowers, so they are also likely exposed to contaminants through there feeding regime. Mysid shrimp tolerate a wide range of salinities.	
Limitations	Amphipod crustaceans are more commonly used for short term sediment bioassays. They are considered benchmark species by the USEPA and USACE Inland Testing Manual (1998) in that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	USEPA. 1995. Environmental Monitoring and Assessment Program Laboratory Methods Manual, Estuaries, Volume 1-Biological and Chemical Analyses, EPA/620/R-95/008. Environmental Monitoring and Assessment Program , U.S. Environmental Protection Agency, Washington DC.	
Website	http://www.epa.gov/emap/html/pubs/docs/groupdocs/estuary/field/lab_man.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.2.3-10
Method Title	Acute Marine Amphipod Crustacean Sediment Bioassay, EPA Method 100.4
Purpose	This test measures the survival of the marine amphipod crustaceans, *Ampelisca abdita*, *Eohaustorius estuarius*, *Leptocheirus plumulosus* or *Rhepoxynius abronius*, after exposure to sediments for 10 days in the laboratory.
Method Summary	Infaunal amphipods*, Ampelisca abdita, Eohaustorius estuarius, Leptocheirus plumulosus* and *Rhepoxynius abronius* are used in toxicity studies assessing sediments from marine and estuarine environments. The toxicity test is conducted for 10 d in 1-L glass chambers containing 175 mL sediment and about 800 mL of overlying water. Test temperature is 15°C for *E. estuarius*, 20°C for *A. Abdita* and 25°C for *L. plumulosus*, and the recommended overlying water salinity is 20 ppt for *E. estuarius* and *L. plumulosus* and 28 ppt for *A. abdita* and *R. abronius*. There will be no feeding during the test and no renewal of overlying water. The endpoints in the toxicity test are survival of amphipods. Performance criteria established for this test include the average survival of amphipods in negative control treatment must be ≥ 90%. The *Rhepoxynius abronius* is the preferred species for coarser-grained sediments (*i.e.,* fines <60%) with a salinities >25 ppt. The *Ampelisca abdita* may be used when test sediment contains > 60% fines and in a wide range of salinities. The *Eohaustorius estuarius* may be used when grain size ranges from 0.6 % sand to 100% sand and salinities range from 1 ppt to 25 ppt. Puget Sound Dredged Disposal Analysis Program (USACE/WDNR/WDEC, 2000) also recommends a similar 10-day acute toxicity test with the marine amphipods, *Ampelisca abdita, Eohaustorius estuarius,* and *Rhepoxynius abronius*. ASTM E1367 and NHEERL-AED SOP 1.03.002 also describe methods for sediment bioassays with amphipods (ASTM, 2001b and USEPA and Naval Construction Battalion Center, 1992, respectively). A miniaturized method of this test also exists (Ho, 2000) and will be incorporated into EPA's TIE guidance document.
Data Uses/Application	This is a sediment toxicity method used to evaluate the effects (reduction in survival) of marine and estuarine sediments on the marine amphipods. The test method may be useful in assessing sediment contamination, registration of pesticides, assessments of new and existing chemicals, Superfund site assessment, and assessment and cleanup of hazardous waste treatment, storage, and disposal facilities. The choice of these amphipod species as test organisms is based on sensitivity to sediment-associated contaminants, availability and ease of collection, tolerance of environmental conditions (*e.g.*, temperature, salinity, grain-size), ecological importance, and ease of handling in the laboratory. Either alone or in combination they may be used to measure toxicity of any commonly encountered marine sediment.

Fact Sheet No.	2.2.3-10 (contd.)	
Advantages	Amphipods are among the first taxa to disappear from benthic communities impacted by pollution, and have been shown to be more sensitive to contaminated sediments than several other major taxa. All of these organisms are considered benchmark species by the USEPA and USACE's Inland Testing Manual (1998) indicating that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species.	
Limitations	Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	USEPA. 1994d. Methods for Assessing the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Amphipods, EPA/600/R-94/025. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/waterscience/cs/freshmanual.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.2.3-11	
Method Title	Acute Marine Polychaete Sediment Bioassay, ASTM Method E1611-00	
Purpose	This test measures the survival of the marine polychaetes, *Neanthes arenaceodentata* and *Neanthes virens*, after exposure to sediments for 10 days in the laboratory.	
Method Summary	Infaunal polychaetes *Neanthes arenaceodentata and Neanthes virens* are used in toxicity studies assessing sediments from marine and estuarine environments. The toxicity test is conducted for 10 d in 1-L glass chambers with a sediment depth of 2 to 3 cm and aerated overlying water. Water is not renewed during the 10-day exposure and there is no feeding. The endpoints in the toxicity test is survival of polychaetes. A negative control or reference sediment is used to give a measure of acceptability of the test. A similar method using the test species *Polydora cornuta* is described in Environment Canada's method EPS/1/RM/41	
Data Uses/Application	This is a sediment toxicity method used to evaluate the acute effects (reduction in survival) of marine and estuarine sediments on polychates. Polychaetes are an important component of the benthic community and are sensitive to both organic and inorganic chemicals. The results of this acute toxicity test can be used to predict temporal or spatial distribution of sediment toxicity. The test method may be useful in assessing sediment contamination, registration of pesticides, assessments of new and existing chemicals, Superfund site assessment, and assessment and cleanup of hazardous waste treatment, storage, and disposal facilities.	
Advantages	Polychaetes are burrowers and deposit-feeders; therefore, contaminant exposure is likely. A 10-day test provides data on the short-term effects that may be useful for comparisons to other species but does not provide information on delayed effects.	
Limitations	Polychaetes are not considered benchmark species (USEPA and USACE, 1998). Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	ASTM. 2001b. ASTM Book of Standards. Volume 11.05. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.3-12	
Method Title	Chronic Estuarine Amphipod Sediment Bioassay	
Purpose	This test measures the growth and reproduction of the estuarine amphipod crustacean, *Leptocheirus plumulosus,* after exposure to sediments for 28 days in the laboratory.	
Method Summary	This is a laboratory method for determining the chronic toxicity of contaminants associated with whole sediments collected from estuarine or marine environments (or estuarine or marine sediment spiked with compounds in the laboratory). The toxicity method uses an estuarine crustacean, the amphipod, *Leptocheirus plumulosus*. The toxicity test is conducted for 28 d in 1-L glass chambers containing 175 mL sediment and about 725 mL of overlying water. Test temperature is 25°± 2°, and the recommended overlying water salinity is 5 ppt ± 2ppt (for test sediment with pore water at 1 ppt to 10 ppt) or 20 ppt ± 2 ppt (for test sediment with pore water >10 ppt). 400 mL of overlying water is renewed three times a week, at which times test organisms are fed. The endpoints in the toxicity test are survival, growth, and reproduction of amphipods. Performance criteria established for this test are that the average survival of amphipods in negative control treatment must be ≥ 80% and there must be measurable growth and reproduction in all replicates of the negative control treatment.	
Data Uses/Application	This is a sediment toxicity method used to evaluate the sublethal effects (reduction in growth and reproduction) of marine and estuarine sediments on the marine amphipod, *Leptocheirus plumulosus*. The test method may be useful in assessing sediment contamination, registration of pesticides, assessments of new and existing chemicals, Superfund site assessment, and assessment and cleanup of hazardous waste treatment, storage, and disposal facilities.	
Advantages	The marine amphipod, *Leptocheirus plumulosus,* is considered a benchmark species which means that they comprise a substantial database, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species. This organisms is a deposit-feeder and burrower therefore exposure to sediment contaminants will occur through its feeding regime.	
Limitations	The test is applicable for use with sediments from oligohaline to fully marine environments, with a silt content greater than 5% and a clay content less than 85%. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	USEPA. 2001e. Method for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-associated Contaminants with the Amphipod *Leptocheirus plumulosus,* EPA 600/R-01/020. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/waterscience/cs/guidancemanual.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.2.3-13	
Method Title	Chronic Marine Polychaete Sediment Bioassay, ASTM Method E1611-00	
Purpose	This test measures the growth and reproduction of the marine polychaete, *Neanthes arenaceodentata*, after exposure to sediments for 28 days in the laboratory.	
Method Summary	This is a laboratory method for determining the chronic toxicity of contaminants associated with whole sediments collected from estuarine or marine environments (or estuarine or marine sediment spiked with compounds in the laboratory). The toxicity method uses an estuarine polychaete, *Neanthes arenaceodentata*. The toxicity test is conducted for 28 d in 1-L glass chambers containing 2-3 cm of sediment and aerated overlying water. This is a static renewal toxicity test and the organisms are fed daily. The endpoints in the toxicity test are survival, growth, and reproduction of amphipods. Performance criteria established for this test include the average survival of amphipods in negative control treatment must be ≥ 80% and there must be measurable growth and reproduction in all replicates of the negative control treatment. Puget Sound protocols and guidelines recommend a juvenile polychaete bioassay with a 20-day exposure time. Endpoints are mortality, total biomass, and average individual biomass. It is a static renewal assay and organisms are fed.	
Data Uses/Application	This is a sediment toxicity method used to evaluate the sublethal effects (reduction in growth and reproduction) of marine and estuarine sediments on the marine polychates. The test method may be useful in assessing sediment contamination, registration of pesticides, assessments of new and existing chemicals, Superfund site assessment, and assessment and cleanup of hazardous waste treatment, storage, and disposal facilities.	
Advantages	This type of worm is a deposit-feeder and burrower, therefore exposure to sediment contaminants is likely through the feeding regime.	
Limitations	This organism is not considered a benchmark species (USEPA and USACE, 1998). The protocol may have to be modified for tests at salinities less than 20 ppt. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	ASTM. 2001b. ASTM Book of Standards. Volume 11.05. American Society for Testing and Materials, West Conshocken, PA.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.2.3-14	
Method Title	Ames Mutagenicity Assay	
Purpose	This test can be used to determine if a sample is mutagenic or cancer-causing. It uses the induced reversion of bacterial mutants to detect DNA-damaging substances.	
Method Summary	Sediment samples are dried with anhydrous sodium sulfate, extracted with methylene chloride, subjected to gel-permeation chromatography cleanup, evaporated under nitrogen, and brought to volume in DMSO. The Ames assay uses 100uL of cultured test strain (*Salmonella typhimurium*), 500 uL of either phosphate buffer, and 100uL of the sediment extract or DMSO mixture (control). The entire mixture is incubated for 20 to 30 minutes in a dry block heater. Following incubation, top agar is added containing trace histidine and biotin and the mixture is poured into plates. Plates are then incubated and the resulting colonies are counted at 72 hours. A positive mutagenic response is indicated when the number of revertants on test plates are greater than or equal to 2 times the number of colonies in the DMSO solvent control plate.	
Data Uses/Application	Short-term bioassays generally identify specific genotoxic contaminants or those in complex mixtures of contaminants, provide baseline data for monitoring changes in environmental conditions, and predict potential long range genotoxic health effects.	
Advantages	The Ames test is relatively quick and inexpensive. It is useful for establishing priorities for more definitive chemical analysis or toxicological testing.	
Limitations	Some chemicals that are mutagenic/carcinogenic, do not give a positive Ames Test (*i.e.,* dioxin). Because the strain of salmonella used is histidine negative, the test may also give false positives if histidine is present in environmental samples. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	USEPA. 1993b. Assessment and Remediation of Contaminated Sediments (ARCS) Program: Biological and Chemical Assessment of Contaminated Great Lakes Sediment, EPA 905-R93-006. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/arcs/EPA-905-R93-006/EPA-905-R93-006.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.2.3-15	
Method Title	Mutatox Genotoxicity Assay	
Purpose	This test can be used to determine if a sample is mutagenic or cancer-causing. It uses the induced reversion of bacterial mutants to detect DNA-damaging substances.	
Method Summary	Sediment samples are dried with anhydrous sodium sulfate, extracted with methylene chloride, subjected to gel-permeation chromatography cleanup, evaporated under nitrogen, and brought to volume in DMSO. The Mutatox assay uses rat liver S9 for exogenous metabolic activation of progenotoxins and a dark mutant strain of the luminescent bacterium *Photobacterium phosphoreium* for detection of genotoxins. DNA-damaging substances are detected by measuring the ability of a test extract or specific chemical to restore the luminescent state in the bacterial cells. The degree of light increase indicates the relative genotoxicy of the sample.	
Data Uses/Application	Short-term bioassays generally identify specific genotoxic contaminants or those in complex mixtures of contaminants, provide baseline data for monitoring changes in environmental conditions, and predict potential long range genotoxic health effects.	
Advantages	The test is relatively quick and inexpensive. It is useful for establishing priorities for more definitive chemical analysis.	
Limitations	Comparative data may be limited. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	USEPA. 1993b. Assessment and Remediation of Contaminated Sediments (ARCS) Program: Biological and Chemical Assessment of Contaminated Great Lakes Sediment, EPA 905-R93-006. Great Lakes National Program, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/arcs/EPA-905-R93-006/EPA-905-R93-006.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.2.3-16
Method Title	V79 Sister Chromatid Exchange Assay, NHEERL-AED SOP 1.03.012
Purpose	These methods describes a test used to evaluate mutagenic effects of single compounds and complex mixtures, including sediment extracts and their fractions.
Method Summary	The Sister Chromatid Exchange (SCE) first requires the preparation of media that is used for plating, dosing and media change procedures. The media is then divided and applied to toxicity plates and SCE plates. Cell suspensions are prepared and then added to appropriate vials in a 1:10 dilution ratio. The number of cells per mL in the cell suspensions are determined using a hemocytometer. Cell suspension dilutions are then added to the toxicity plates and the SCE plates. These plates are then incubated for 24 hours to allow for cell attachment. Following incubation, media and solvents are mixed to prepare a dosed medium that is then added to both the toxicity plates and the SCE plates: the toxicity plates receive a minimum of 3 mL of dosed media and the SCE plates receive a minimum of 7 mL of dosed media. The plates are then incubated for 5 hours. Media is renewed with "clean media" on the toxicity plates and then the plates are returned to the incubator for 6 days. The media is changed again and the plates are incubated for 20 hours. Cells are harvested from both the toxicity plates and the SCE plates and slides are made. On the toxicity slides, the number of colonies are counted. The SCE slides are scanned for suitable chromosome spreads. The number of chromosomes and the number of SCEs per spread are recorded, along with other observations.
Data Uses/Application	The SCE Assay is commonly used to determine genetic damage and mutational events by cytogenetic analysis. The mean colony per dose determined on the toxicity test slides is used to find the percent survival of a dose as compared to the control or blank for that test. The number of SCE/number of chromosomes is evaluated statistically to find the mean per dose which is compared to the control or blank for that test.
Advantages	The assay has been used to evaluate effects of single compounds (*i.e.,* mitocmycin C, benzo(a)pyrene) and to evaluate whole sediment extracts and sediment fractions from sites with known contaminated sediment problems.
Limitations	The tests need to be monitored daily since contamination will skew the results. Any contaminated plates should be removed, taped with autoclave masking tape, wrapped in foil, and autoclaved. There may also be problems with chromosome, staining and spreading quality. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.

Fact Sheet No.	2.2.3-16 (contd.)	
Reference	USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296, Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.03.012.pdf	Last Accessed:

Fact Sheet No.	2.2.3-17
Method Title	Bioaccumulation Test for Marine, Estuarine and Freshwater Sediments, EPA Method 100.3
Purpose	This test measures bioaccumulation in the freshwater *Lumbriculus variegatus* after exposure to sediments for 28 days in the laboratory.
Method Summary	The *Lumbriculus variegatus* is exposed to sediments for 28-days in a 4 to 6 L container containing 1 to 2 L of sediment and 1 to 4 L of overlying water. Test temperature is 23 +/- 1 °C. 1 L of overlying water will be renewed every 12 hours. No supplemental food will be added during the experiment. The endpoint is bioaccumulation. In some cases, body burdens will not approach steady-state body burdens in a 28-d test (*e.g.*, organic compounds with a log Kow >5, be metabolically refractory, or have low depuaration rates). Depending on the goals of the study and the adaptability of the test species to long-term testing, it may be necessary to conduct an exposure longer than 28-d (or a kinetic study) to obtain a sufficiently accurate estimate of steady-state tissue residues of these compounds. Use of long-term tests or toxicokinetic approaches is recommended specifically for slowly accumulated compounds and for a greater than 80 percent accuracy in test species achieving steady state. ASTM also provides guidance for bioaccumulation tests with marine test species such as the polychaetes, *Nereis diversicolor*, *Neanthes virens* and the bivalve *Macoma nasuta*, *Macoma balthica*, and *Yoldia limatula* (ASTM Method E1688; ASTM 2001b), and freshwater test species such as the *Diproeia spp* and *Lumbriculus* variegates. They recommend selecting at least one species representing filter-feeding, deposit-feeding and burrowing species.
Data Uses/Application**	This is a laboratory method for determining the bioaccumulation of contaminants associated with sediments collected from freshwater environments. The data are sometimes multiplied by an adjustment factor to accommodate for different steady-state rates with different contaminant mixtures. Data from bioaccumulation tests are used to derive bioaccumulation factors (BAFS) and to determine biota-sediment accumulation factors (BSAFS) in equilibrium partitioning models. Bioaccumulation data is needed in ecological or human health risk assessments, therefore the procedures are designed to generate quantitative estimates of steady-state tissue residues. These tests are also used to assist in the development of sediment quality criteria and to assess the potential impacts of disposal of dredged materials. Biota-sediment accumulation factors (BSAFS) are often compared between laboratory-exposed and field-exposed organisms to determine the validity of laboratory experiments and to better predict contaminant-specific lipid partitioning tendencies.

Fact Sheet No.	2.2.3-17 (contd.)	
Advantages	Previous bioaccumulation tests used 10-day exposures. A 28-day exposure is a practical compromise between cost, data accuracy and data utility. Observed steady-state tissue levels were reached in 28-days in 69% of the tests. The data should be sufficiently accurate for quantitative risk analysis in most cases. In cases in which more accurate estimates are required, either a long-term exposure or an alternative approach can be used.	
Limitations	Additional research is needed on the standardization of bioaccumulation procedures with sediment. Steady-state is reached at different times with different contaminants, thus 28-day is an approximate period of time that may prove inaccurate with the wrong mixture of contaminants. Specifically, the 28-day time period appears to underestimate steady-state of DDT and dieldrin, so it may be necessary to use adjustment factors for sites with significant DDT and dieldrin concentrations. Please note the list of general limitations for all solid-phase toxicity tests in the introduction to this section.	
Reference	USEPA. 2000a. Bioaccumulation Testing and Interpretation for the Purpose of Sediment Quality Assessment: Status and Needs, EPA 823-R-00-001. Office of Water and Office of Solid Waste, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html	Last Accessed: 2/13/03

2.3 Biota

The health and community structure of endemic organisms are often evaluated in order to assess the impacts of chemical contamination on the environment. At Superfund sites containing contaminated sediments, phytoplankton, zooplankton, benthic invertebrates and fish can all be monitored. These organisms can potentially be exposed to contaminant stressors via ingestion pathways or direct contact/absorption from the water column. Therefore, they are collected either for identification and enumeration analyses to determine community structure or they are collected for chemical tissue analyses to investigate contaminant uptake, bioaccumulation and potential biomagnification in the food chain.

There are three common approaches to evaluating environmental risk to receptors: 1) the use of literature screening values; 2) a "desk-top" risk assessment that can model existing site-specific contaminant data to ecological receptors for subsequent comparison to literature toxicity values; or 3) field investigation/laboratory analysis that involves a site investigation and laboratory analysis of contaminant levels in media and/or experimentation using bioassay procedures (USEPA, 1997a). The methods provided in the following section provide a summary of those methods that would be used in the third approach: field investigation and laboratory analyses. These fact sheets intend to provide Superfund managers with a summary of the existing methods that may be applicable to their site, the method's relative strengths, and the method's relative weaknesses. These analyses will help to determine the relationship between the exposure of a contaminant and the response it elicits.

2.3.1 Chemical and Physical Analyses

Section 2.3.1 presents field sample collection and processing methods for biota. Biota are collected at Superfund sites for chemical residue studies, population/community studies, and toxicity testing/bioassays; all directed at assessing exposure-response relationships at the site (USEPA, 1997a). Methods are provided for sample collection and processing of phytoplankton, zooplankton, periphyton, benthic invertebrates and fish. Biota methods were predominantly gathered from the following sources:

- The USEPA's Office of Water
- The USEPA's EMAP program
- The USEPA's Great Lakes Program

Fact Sheet No.	2.3.1-1	
Method Title	Phytoplankton Sample Collection and Preservation in the Great Lakes, LMMB 023t	
Purpose	This method describes the collection and preservation of phytoplankton samples for community analyses	
Method Summary	Water samples are collected using a Rosette sampler (see Fact Sheet 2.1.1-6). 1L aliquots from each discrete sampling depth are composited, and approximately 1 L of the composite sample is transferred to a sample bottle. The sample is preserved with Lugol's Solution for analysis (final concentration 1%). Samples are stored in the dark and refrigerated.	
Data Uses/Application	Phytoplankton community analyses are useful for bioassessments to determine community disturbance as a result of contamination.	
Advantages	One of few standard methods for water column bioassessment	
Limitations	This collection method is not suitable for chlorophyll *a* or productivity measurements. Populations of phytoplankton are seasonal and highly variable. Use of one or more reference stations is essential.	
Reference	USEPA. 1997b. Method LMMB 023: Standard Operating Procedure for Phytoplankton Sample Collection and Preservation. Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/lmmb/methods/phytocol.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-2	
Method Title	Chlorophyll-*a* Sampling Method and Preservation: Field Procedure in the Great Lakes, LMMB 015	
Purpose	This method is used to filter chlorophyll-*a* samples.	
Method Summary	Water samples are collected using Niskin bottles or other suitable sampling device. The water samples are transferred from the Niskin bottles to opaque sample bottles for storage. The water sample for chlorophyll-*a* analysis is vacuum filtered through a 47 mm diameter glass fiber filter (see Fact Sheet 2.3.1-3). The entire procedure is conducted in subdued (green) light to prevent photodecomposition. During filtration, the samples are treated with a solution of $MgCO_3$ to prevent acid induced transformation of chlorophyll to its degradation product, Phaeophytin. Sample filters are stored in aluminum foil pouches and frozen until analysis.	
Data Uses/Application	Often used as a surrogate for productivity or standing crop measurements, chlorophyll *a* measurements are also used to monitor plankton blooms. Also used to calibrate SeaWiFS or other remote sensing images.	
Advantages	Simple collection, extraction, and analysis methods allow economical spatial and temporal variations to be monitored.	
Limitations	Filters must be extracted and analyzed within 28 days.	
Reference	USEPA. 1997b. Method LMMB 015: Standard Operating Procedure for Chlorophyll-*a* Sampling Method: Field Procedure. Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/lmmb/methods/chlfield.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-3	
Method Title	Chlorophyll *a* and Phaeophytin Field Filtering Protocols	
Purpose	This method describes the methods used for the immediate processing of water samples analyzed for Chlorophyll *a* and Phaeophytin.	
Method Summary	Chlorophyll *a* and Phaeophytin samples must be filtered no more than 4 hours after collection. Samples that cannot be filtered immediately after collection must be held at 4°C until filtered. Filtering can be accomplished by the use of a vacuum pump or by using positive pressure, as described below Vacuum Filtration: Filter the water onto two 47 mm diameter glass fiber filter pads. Do not exceed a vacuum of 15 psi or a filtration duration of greater than 5 minutes. Add 1 mL of saturated $MgCO_3$ solution during the last few seconds of filtering after the nutrient filtrate has been removed. Record the volume filtered on the data sheet. The filtrate should be saved for dissolved nutrient analyses. Approximately 40 mL of filtrate will be collected into a pre-labeled, clean 60 mL Nalgene screw-capped bottle and stored on dry ice. Carefully remove the filters using forceps, fold in half, and wrap in aluminum foil. Label the samples and place package on dry ice. Positive Pressure: The alternative method is to use positive pressure to push a sample through the filter. A disposable, graduated 50-cc polypropylene syringe fitted with a stainless steel or polypropylene filtering assembly is used to filter the site water through 25 mm diameter glass fiber filter pads; the volume of water must be documented. If conditions allow, up to 200 mL of site water should be filtered for each chlorophyll sample. After filtering, add 1 mL of $MgCO_3$ solution to the syringe and pass through the filter pad. Remove the filter, fold and place in aluminum foil. Again, approximately 40 mL of filtrate will be collected into a pre-labeled, clean 60 mL Nalgene screw-capped bottle and stored on dry ice. Methods LMMB 085 and 086 describe similar filtering protocols (USEPA, 1997b).	
`Data Uses/Application	Chlorophyll *a* measurements are indicative of the primary producer's relative abundance and composition in the water column sample.	
Advantages	Simple collection, extraction, and analysis methods allow economical spatial and temporal variations to be monitored.	
Limitations	If filtration cannot occur in under 4 hours, the phytoplankton cells can possibly lyse. The sample on the filter paper may degrade over time, and must be extracted and analyzed within 28 days.	
Reference	USEPA. 2000b. Coastal 2000 Northeast Component: Field Operations Manual, Environmental Monitoring and Assessment Program (EMAP), EPA/620/R-00/002. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.3.1-4	
Method Title	Primary Productivity Using ^{14}C: Field Procedure in the Great Lakes, LMMB 016	
Purpose	This method is used to determine primary productivity and primary productivity parameters from water.	
Method Summary	Water samples are collected using Niskin bottles or other suitable samplers. When there is a thermal stratification in the water column, samples are collected from both the hypolimnion and the epilimnion. The water samples are transferred from the Niskin bottles to opaque sample bottles for storage in a light-tight, insulated container. The following procedures are conducted in subdued (green) light to avoid photodegradation. Water samples are carefully transferred to incubation bottles. Water samples are inoculated with a known quantity of bircarbonate substrate, which is labeled with the radiotracer ^{14}C. Samples are incubated at various light intensities for 2 - 4 hours. After incubation, a 100 mL aliquot of each sample is filtered through a 47 mm cellulose acetate filter (0.45 µm pore size). The filter is placed into a scintillation vial and 0.5 N HCl is added. The vials sit at room temperature for 1 hour. 20 mL of liquid scintillation cocktail is added to each vial. The vials are stored until they are analyzed by liquid scintillation counting to determine the quantity of carbon fixed by the algae into organic matter.	
Data Uses/Application	Primary productivity is a key measurement in many site assessments, particularly those affected with nutrient enrichment.	
Advantages	This method is the standard for productivity determination.	
Limitations	Use of ^{14}C requires NRC License. Disposal of ^{14}C waste is often problematic.	
Reference	USEPA. 1997b. Method LMMB 016: Standard Operating Procedure for Primary Productivity Using ^{14}C: Field Procedure. Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/lmmb/methods/c14field.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-5	
Method Title	Zooplankton Sample Collection and Preservation in the Great Lakes, LMMB 024	
Purpose	This method describes the collection and preservation of zooplankton samples for community analyses.	
Method Summary	Water samples are collected using a plankton tow net that is maneuvered using a winch on the starboard side of the vessel. The tow net has a flow meter and screened sample bucket attached to the end. The flow meter should be calibrated every survey season. For sampling, the net is lowered to the desired depth (usually 20 meters from the water surface) and raised at a constant slow speed until the rim is above the water. In shallower waters, the samples are usually collected from 1 meter above the bottom to the surface. The net is then lifted out of the water and rinsed from the outside to free organisms from the side of the net, concentrating them into the sample bucket. The sample bucket is removed, and the sample is rinsed into a sample container. The organisms are then narcotized with 20 mL of soda water and left to sit for 30 minutes. The samples are preserved with 20 mL of formalin solution.	
Data Uses/Application	Tow nets survey the biological community by collecting abundance and taxa composition data from sampling locations. They are often used to gain information on particular species of larval fish and an overall estimation of fish populations and communities. Fish population and community data are used to measure the status and trends of environmental pollution freshwater, estuarine and marine organisms to assess water quality criteria and to monitor surface water quality.	
Advantages	The tow net is easy to handle and it is small enough for use on boats 4 m or larger in length. The design reduces current vibrations in the water directly in front of the net.	
Limitations	Zooplankton populations are highly seasonal and within season can vary spatially depending upon currents and microclimate. Effective use of reference areas required.	
Reference	USEPA. 1997b. Method LMMB 024: Standard Operating Procedure for Zooplankton Sample Collection and Preservation. Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/lmmb/methods/zoofld.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-6	
Method Title	Field-based Periphyton Survey in Wadeable Streams	
Purpose	To perform a quick semi-quantitative assessment of algal biomass and taxonomic composition in the field.	
Method Summary	This protocol describes a field-based rapid survey of periphyton biomass and coarse-level taxonomic composition (*e.g.*, diatoms, filamentous greens, blue-green algae). To perform a field-based periphyton survey, the investigator establishes three transects across the habitat being sampled (preferably riffles or runs in the reach in which benthic algal accumulation is readily observed and characterized). Three locations are then selected along each transect. Algae are characterized in each of the selected locations by immersing a bucket with a 50-dot grid in the water. Macroalgal biomass and microalgal cover are then determined in the bucket. EPA's EMAP field document describes similar field-based periphyton surveys (USEPA, 1998, EPA/620/R-94/004F).	
Data Uses/Application	Species relative abundance and taxa richness are data derived from these protocols. These data parameters provide information pertaining to the status and trends of environmental pollution and its impacts on freshwater, marine and estuarine communities. Biological impairment resulting from pollution is often evaluated using metrics of biotic integrity derived from the aforementioned data parameters that evaluate community, population and functional parameters. Examples of metrics based on species composition include species richness, total number of genera, total number of divisions, shannon diversity (for diatoms), percent community similarity of diatoms, pollution tolerance index for diatoms, and percent sensitive diatoms. Furthermore, other metrices infer ecological conditions based on documented preferences. These metrices include the percent aberrant diatoms, percent motile diatoms, simple diagnostic metrics, inferred ecological conditions with simple autecological indices (SAI), inferred ecological conditions with weighted average indices, and impairment of ecological conditions.	
Advantages	The field-based periphyton survey requires less effort than the laboratory methods. It is able to assess algal biomass over larger spatial scales than substrate sampling and laboratory analysis. Coarse-level taxonomic characterization of communities is also possible with this technique.	
Limitations	The field methods are not as accurate as the laboratory analysis.	
Reference	Barbour et al. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition, EPA 841-B-99-002. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/owow/monitoring/rbp/	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-7	
Method Title	Laboratory-Based Periphyton Survey: Single Habitat Sampling in Wadeable Streams	
Purpose	To determine the periphyton abundance and composition in area of interests.	
Method Summary	Single habitat sampling protocol outlines a procedure for collecting periphyton from a single substrate/habitat combination that characterizes the study reach. A preliminary, visual habitat assessment should be performed prior to sample collection to determine the percent cover of each substrate type and the estimated relative abundance of organisms. Collection techniques depend on the substrate type and the dominant periphyton. Several subsamples should be collected from the same substrate/habitat combination and composited into a single container. Periphyton samples should be collected during periods of stable stream flow. If the samples are going to be assayed for chlorophyll a, the samples should not be preserved until they have been subsampled. Following subsampling and preservation (Lugol's solution, "M3" fixative, buffered 4% formalin, 2% glutaraldehyde, or other preservative) the samples are transported back to laboratory on ice. EPA's EMAP field document describes similar protocols for periphyton surveys in wadeable streams (USEPA, 1998).	
Data Uses/Application	Periphyton can be collected to: 1) determine taxonomic composition and relative abundance, 2) determine chlorophyll, 3) determine biomass, and 4) determine acid/alkaline phosphate activity. Single habitat sampling provides periphyton biomass data.	
Advantages	Variability in habitat differences between streams may be reduced if periphyton collection is performed from a single substrate/habitat combination.	
Limitations	Spatial variability can lead to samples not being representative of site. Adequate replication and spatial coverage required.	
Reference	Barbour et al. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. Office of Water;\\, U.S. Environmental Protection Agency; Washington, D.C.	
Website	http://www.epa.gov/owow/monitoring/rbp/	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-8	
Method Title	Laboratory-Based Rapid Periphyton Survey: Multi habitat Sampling in Wadeable Streams	
Purpose	Algae sampling methods for subsequent laboratory assessments of species composition.	
Method Summary	Multi-habitat sampling is a procedure developed to sample periphyton from wadeable streams. It should be conducted at the reach scale (30-40 stream widths) to ensure sampling the diversity of habitats that occur in the stream. The protocol first calls for visual estimates or quantitative transect-based assessments to determine the percent cover of each substrate type and the estimated relative abundance of macrophytes, macroscopic filamentous algae, diatoms and other microscopic algal accumulations, and other biota. Following preliminary investigations, algae are collected from all available substrates and habitats roughly in proportion to their areal coverage in the reach. Periphyton samples should be collected during periods of stable stream flow. Small amounts of subsample (about 5 mL or less) are usually sufficient. The objective is to collect a single composite sample that is representative of the periphyton assemblage present in the reach. Collection techniques depend on the substrate type and the dominant macroinvertebrates. However, this protocol recommends that specimens of macroalgae be collected by hand in proportion to their relative abundance in the reach. Samples are combined into single, water-tight, unbreakable, wide-mouth containers. After adding the appropriate preservative, (Lugol's solution, "M3" fixative, buffered 4% formalin, 2% glutaraldehyde, or other preservative), the samples are transported back to laboratory on ice. EPA's EMAP field document describes similar protocols for periphyton surveys in wadeable streams (USEPA, 1998).	
Data Uses/Application	Multi-habitat sampling will provide information pertaining to species composition. Changes in species composition among habitat are often evident as changes in color and texture of the periphyton. These data provide information pertaining to the effects of pollution on environmental communities. Multi habitat sampling can also be conducted to collect periphyton for chlorophyll determination, biomass determination and acid/alkaline phosphate activity.	
Advantages	The investigators may get a better sense of how habitat changes may impact different benthic communities.	
Limitations	There may be variability of data due to differences in habitat between streams. The results may not be sensitive to subtle water quality changes because of habitat variability between reaches.	
Reference	Barbour et al. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition, EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.	
Website	http://www.epa.gov/owow/monitoring/rbp/	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-9
Method Title	Artificial Substrate Samplers of Macroinvertebrates in Wadeable Streams
Purpose	These methods describe the use of artificial substrate samples that have long been used in algal investigations and in situations where bottom substrate sampling is not possible due to physical obstacles. The Rapid Bioassessment Protocols (RBPs) list artificial substrates as a sampling methodology to collect both periphyton and benthic macroinvertebrates.
Method Summary	Artificial substrate samplers typically use glass slides as substrate, but also are deployed with glass rods, ceramic tiles and other substances. The samplers are positioned in the euphotic zone of good light penetration for maximum abundance and diversity of macroinvertebrates. Optimum time for substrate colonization is six weeks. At least two to three samplers should be installed at each collecting site. To retrieve the samplers, they are approached from downstream, lifted quickly and placed in a polyethylene jug or bag containing 10% formalin or 70-80% ethanol. The organisms can be removed in the field by disassembling the sampler in a tub or bucket partially filled with water and scrubbing the rocks or plates with a soft-bristle brush to remove clinging organisms. The contents of the bucket are poured through a No. 30 or 60 sieve and the contents of the sieve are washed into a jar and preserve with 10% formalin or 70-80% ethanol. The use of artificial substrate samplers in macroinvertebrate field and laboratory studies is also presented in USEPA, 1990b.
Data Uses/Application	The benthic composition and abundance data from artificial substrate samplers are used to measure the status and trends of environmental pollution, and effects on freshwater, estuarine, and marine macroinvertebrates, and to assess surface water quality. The RBP protocols caution that artificial substrates should only be used for benthic macroinvertebrates when other collection devices fail. The substrate used must be representative of the natural habitat.
Advantages	Artificial substrates allow sample collection in locations that are typically difficult to sample effectively. As a passive sample collection device, artificial substrates permit standardized sampling by eliminating subjectivity in sample collection techniques. Sample collection using artificial substrates may require less skill and training than direct sampling of natural substrates.

Fact Sheet No.	2.3.1-9 (contd.)	
Limitations	The limitations commonly encountered when using the artificial substrate sampler include susceptibility to vandalism, sampling bias for insects, difficulty in anchoring the device, and the lengthy time from initiation to conclusion of sampling (up to 8 weeks). Furthermore, the material of the substrate will influence the composition and structure of the community. Orientation and length of exposure of the substrate will influence the composition and the structure of the community.	
Reference	Barbour, et al. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.	
Website	http://www.epa.gov/owow/monitoring/rbp/	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-10	
Method Title	Algae and Macroinvertebrate Sampling with Frames	
Purpose	To delineate the percent coverage of the colonial forms of algae and macroinvertebrates.	
Method Summary	A 0.1 m^2 or 1m^2 square-shaped metal frame can be laid flat along rocky shores, beaches etc. and be used to delineate the percent coverage of colonial frames. At least ten frames should be used for counting organisms to characterize species abundance and distribution adequately. Samples of the algae and macroinvertebrates should be removed from a measured area for species identification and weighed for biomass determination.	
Data Uses/Application	An investigator can gather macrobenthos data using frames from locations where conventional sampling devices are not practical. Macrobenthos data are used in benthic community analyses to measure the status and trends of environmental pollution, and its effects on freshwater, estuarine, and marine macroinvertebrates, and to assess water quality criteria and monitor surface water quality.	
Advantages	This method is useful for sampling beach infauna.	
Limitations	Frames delineate organisms present on the surface, however all organisms burrowed beyond the investigator's line of site will not be counted. Rely upon statistics to derive species abundance and distribution for the entire region.	
Reference	USEPA. 1990b. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA/600/4-90/030. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleORD.html	Last Accessed: 2/13/03

Fact Sheet No.	2.3.1-11	
Method Title	Benthic Organism Collection from a Marine Environment, NHEERL-AED SOP 1.02.001	
Purpose	This procedure describes the methods required to collect infaunal and epibenthic marine organisms for tissue analyses or for use in toxicological evaluation.	
Method Summary	Benthic macroinvertebrates: Sediments are frequently collected with grab samplers, such as the Young-modified Van Veen grab. This particular sampler is constructed entirely of stainless steel and can be Kynar-coated to make it suitable for collecting sediment samples for both biological and chemical analyses. The samples should be numbered and the depth of the sediment at the middle of the sampler should be recorded on the data sheet. The sampler should be at least half full. The data sheet should also include a general description of the grab such as the presence or absence of surface floc, color and smell of surface sediments, and visible fauna. Worms: Sediments from grab samples are emptied into a tub and then passed through sieves. The appropriate sieve size could be selected based on sediment type and organisms to be collected. Worms are picked from the sieves, rinsed free of sediment, and placed in sample jars. Quahogs: Quahogs should be collected with the aid of a professional quahog fisherman. The fisherman should be provided with one extra individual for assistance, a sampling location chart, and prelabelled and organized sample bags. *Mytilus*: Mussels are collected with a scallop dredge towed at 2-3 knots for 5-10 minutes. The catch is then hauled back, dumped on board, and sorted. Oysters: Hand collection in shallow water is recommended. Soft-shell clams: Soft-shelled clams should be collected by hand at low-tide. After locating siphon holes on the tidal region of the flat, the clams can be dug out, taking care to avoid breaking the “soft-shell” during excavation. USEPA’s Coastal and Northeast EMAP document describes similar protocols for benthic organism collection (USEPA, 2000b).	
Data Uses/Application	Benthic organisms can be collected to determine species composition and abundance in a particular sampling reach, or the organisms may be used for tissue residue analyses in toxicological evaluation.	
Advantages	All activities can be performed by hand or from a small boat.	
Limitations	While the use of sediment grab samplers for collecting macroinvertebrates and worms is almost unanimously recommended, the application of other methods is highly site-specific.	
Reference	USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296. Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.	
Website	http://www.duxbury.battelle.org/compendium/methods/NHEERL-AED-SOP-1.02.001.pdf	Last Accessed:
Fact Sheet No.	2.3.1-12	

Method Title	Benthic Macroinvertebrate Protocols in a Wadeable Stream: Single Habitat Approach, 1-Meter Kick Net	
Purpose	To determine macroinvertebrate diversity and abundance in wadeable streams.	
Method Summary	The single habitat approach to benthic macroinvertebrate sampling emphasizes sampling a single, dominant habitat (*i.e.,* riffles or runs) as a way of standardizing assessments. Benthic macroinvertebrate samples are collected systematically using a 1 m kick net. Once the net is in position on the substrate, a rectangular quadrant that is approximately 0.5 m^2 is visually defined. The net is held securely while the substrate is kicked vigorously for 20 seconds. After 20 seconds, the net is removed with a quick upstream motion to wash the organisms to the bottom of the net. The kicks collected from different locations in flowing water habitats will be composited to obtain a single homogeneous sample. Kick net samples collected from pool habitats are combined into a separate composite sample. The percentage of habitat type is recorded along with observations of aquatic flora and fauna and a habitat assessment will be performed. The samples composited from the kick nets will be preserved in 95% ethanol. The samples are then returned to the laboratory for species enumeration and identification. The USEPA EMAP field document describes similar methods for using a 1-m kick net (USEPA, 1998).	
Data Uses/Application	Species relative abundance and taxa richness are data derived from these protocols. These data parameters provide information pertaining to the status and trends of environmental pollution and its impacts on freshwater, marine and Estuarine communities. Biological impairment resulting from pollution is often evaluated using metrics of biotic integrity derived from the aforementioned data parameters that evaluate community, population and functional parameters.	
Advantages	The 1-m kick net method provides a rapid, reproducible, and inexpensive method for the collection of macroinvertebrates from suitable environments.	
Limitations	Single habitats (*i.e.,* cobble substrates) cannot be solely analyzed in reaches where the substrate represents less than 30% of the sampling reach.	
Reference	Barbour et al. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition, EPA 841-B-99-002. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/owow/monitoring/rbp/	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-13	
Method Title	Benthic Macroinvertebrate Protocols in a Wadeable Stream: Multi-habitat Approach: D-Frame Dip Net	
Purpose	To determine macroinvertebrate diversity and abundance in wadeable streams.	
Method Summary	This method focuses on a multi-habitat scheme designed to sample major habitats in proportional representation within a sampling reach. Benthic macroinvertebrates are collected systematically from all available instream habitats by kicking the substrate or jabbing with a D-frame dip net. A total of 20 jabs (or kicks) are taken from all major habitat types in the reach resulting in sampling of approximately 3.1 m^2 of habitat. The samples collected from this protocol will then be sent to the laboratory where species enumeration and identification will be conducted.	
Data Uses/Application	Species relative abundance and taxa richness are data derived from these protocols. These data parameters provide information pertaining to the status and trends of environmental pollution and its impacts on freshwater, marine and estuarine communities. Biological impairment resulting from pollution is often evaluated using metrics of biotic integrity derived from the aforementioned data parameters that evaluate community, population and functional parameters.	
Advantages	It is important to use a multi-habitat approach when the stream under investigation varies in gradient and substrate type.	
Limitations	Differences in sampling techniques can lead to variability and difficulty comparing data among researchers.	
Reference	Barbour et al. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition, EPA 841-B-99-002. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/owow/monitoring/rbp/	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-14	
Method Title	Photographic Habitat Documentation of the Benthic Community	
Purpose	To document a habitat or alterations in a station over time (*e.g.*, increase in canopy cover, changes in channelization of a stream, and effects of flooding etc.)	
Method Summary	Photography of aquatic environments usually involves SCUBA equipment. The SCUBA diver will place a photographically identifiable 1.0 m^2 area frame or marker in the habitat to be photographed and an additional nearby marker on which the camera is placed each time a photograph is taken to ensure consistency.	
Data Uses/Application	Photography is a tool used to characterize benthic composition and potential alterations over time in environments with sessile organisms that may change over time in relation to a new stressor. Photographic images of the abundance and diversity of sessile organisms over time is a way in which to monitor the status and trends of environmental pollution, and its effects on freshwater, estuarine, and marine organisms, and to assess surface water quality.	
Advantages	Photographic documentation is a rapid and inexpensive tool to use to support benthic bioassessments, particularly in areas with significant populations of sessile organisms.	
Limitations	Photography is generally limited to environments with suitably clear water that are inhabited by sessile animals and rooted plants (*e.g.*, estuarine habitats containing corals, sponges, and attached algal forms). Underwater photography generally requires trained SCUBA divers.	
Reference	USEPA. 1990b. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA/600/4-90/030. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleORD.html	Last Accessed: 2/13/03

Fact Sheet No.	2.3.1-15	
Method Title	Sediment Profile Camera	
Purpose	This method describes use of a sediment profile camera to evaluate the *in situ* macroinvertebrate community.	
Method Summary	Photographs are taken of the benthic community prior to any other benthic sampling to avoid disruption of the surface. The sediment profiling index (SPI) system consists of a digital camera enclosed in a waterproof, pressure resistant housing, a 45-degree prism that penetrates the sediment to a depth of 25 cm, and a mirror that reflects an image of the sediment profile through the camera lens and to the digital camera. The camera prism is mounted on an assembly that can be moved up and down by producing tension or slack on the winch wire. As the camera is lowered, tension on the winch wire keeps the prism in the 'up' position until the support frame hits the bottom. At this point the tension on the winch wire is reduced causing the inner frame to move to the 'down' position, penetrating the undisturbed sediment water interface. The upper 25 cm of the sea floor, as seen in profile, is then photographed in high resolution with a film or digital camera. An additional camera mounted on the frame photographs the sediment surface before the prism penetrates the sediment. After each image is taken, the camera is raised two or three meters off the bottom and redeployed for taking another image ('sample').	
Data Uses/Application	SPI cameras are used to evaluate macrofauna community structure and assess the benthic habitat. SPI technology can readily quantify over 20 physical, chemical, and biological parameters including: sediment grain size; prism penetration; surface pelletal layer; sediment surface relief; mud clasts; redox area; redox contrast; current apparent redox boundary; relict redox boundaries; methane gas vesicles; apparent faunal dominants; voids; burrows; surface features (*e.g.*, worm tubes, epifauna, shell); dredged material; microbial aggregations; and successional stage. SPI data have been accepted in the United States by the by the U.S. Environmental Protection Agency and by the U.S. Army Corps of Engineers for describing baseline benthic habitat conditions at proposed dredged material disposal sites, for monitoring changes in sediment structure and the benthic community from dredged material disposal, and for monitoring the recovery of disposal sites and their surrounding environment.	
Advantages	Rapid photographic evidence of sediment conditions.	
Limitations	Provides only a partial picture of benthic community structure, and little information on benthic macroinfauna communities. Generally, limited to screening in soft bottom sediments.	
Reference	USEPA. 1990a. Environmental Monitoring and Assessment Program: Near Coastal Component, 1990 Demonstration Project, Field Operations Manual. DRAFT. Contract # 68-C8-0066. Office of Research and Development. Narragansett, Rhode Island.	
Website	http://www.epa.gov/emap/html/pubs/docs/groupdocs/estuary/field/90fldman.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-16	
Method Title	Macroinvertebrate Drift Nets in Wadeable Streams	
Purpose	To collect macrobenthos inhabiting a wide range of habitat types from shallow flowing streams or shallow areas in rivers for quantitative evaluations.	
Method Summary	For synoptic surveys, one net set above each of the major areas of population concentrations is usually adequate; but for definitive studies a minimum of two drift nets should be set at each station so that drift from above a pollution source, drift from the polluted reach and drift from the clean water downstream from the recovery zone can be compared. Use nets with a 929 cm^2 upstream opening and mesh equivalent to the U.S. Standard No. 30 screen (0.595 mm pore size). Set drift nets for any specified time (usually 3 hours). Sampling between dusk and 1 AM is optimum. For definitive studies, install four nets at each station two about 25 cm from the bottom and tow about 10 cm below the surface in water not exceeding 3 meters in depth. At the end of the specified sampling period, remove the net from the water by loosening the cable clamps and raising the net over the top of the steel rods, taking care not to disturb the bottom upstream of the net. Concentrate the material in the net in one corner by swishing up and down in the water and then wash into a bucket half-filled with water. Then sieve and handle the sample in the regular manner. Standard methods 10500 describe similar collection methods (APHA, 1999).	
Data Uses/Application	Drift nets collect macrobenthos in order to characterize the composition and abundance of macroinvertebrate biota that drift in the water column. A summary of stream net samplers is presented in Table 2.3.1-1. Macroinvertebrate data such as these are used to measure the status and trends of environmental pollution and its effects on freshwater, estuarine, and marine macroinvertebrates, to assess water quality criteria and to monitor surface water quality.	
Advantages	Standard collection method used throughout United States.	
Limitations	It is unknown where the organisms come from; terrestrial species may make up a large part of sample in summer and periods of wind and rain. Drift nets do not collect non-drifting organisms.	
Reference	USEPA. 1990b. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleORD.html	Last Accessed: 2/13/03

Table 2.3.1-1. A Summary of Stream Net Samplers Used to Collect Organisms from Flowing Water (USEPA, 1990b[1])

Net Sampler	Habitats and substrates	Effectiveness of Device	Advantages	Limitations
Surber Stream Bottom Sampler	Shallow, flowing streams, less than 32 cm in depth with good current; rubble substrate, mud, sand and gravel	Performance depends on current and substrate	Encloses area sampled; easily transported or constructed; samples a unit area	Difficult to set in some substrate types, that is large rubble; cannot be used efficiently in still, slow-moving streams
Portable Invertebrate Box Sampler	Same as Surber	Same as Surber	Same as Surber except completely enclosed with stable platform; can be used in weed beds.	Same as Surber
Hess Sampler	Same as Surber	Same as Surber	Same as Surber except completely enclosed with stable platform; can be used in weed beds.	Same as Surber
Hess Stream Bottom Sampler	Same as Surber	Same as Surber	Same as Surber except completely enclosed with stable platform; can be used in weed beds.	Same as Surber
Stream-bed Fauna Sampler	Same as Surber	Same as Surber	Same as Surber except completely enclosed with stable platform; can be used in weed beds.	Same as Surber
Drift nets	Flowing rivers and streams; all substrate types	Effective in collecting all taxa which drift in the water column	Low sampling error; less time, money and effort; collects macroinvertebrates from all substrates, usually collects more taxa	Unknown where organisms come from; terrestrial species may make up a large part of a sample in summer and periods of wind and rain; does not collect non-drifting organisms

[1] USEPA. 1990b. EPA/600/4-90/030.

Fact Sheet No.	2.3.1-17	
Method Title	Stream-Net Samplers: Surber, Portable Invertebrate Box Sampler, Hess Sampler, Hess Stream Bottom Sampler, and Stream-Bed Fauna Sampler	
Purpose	To collect macrobenthos inhabiting a wide range of habitat types from shallow flowing streams or shallow areas in rivers for quantitative evaluations.	
Method Summary	The sampler is positioned with its net mouth open, facing upstream. The samplers are brought down quickly to reduce the escape of rapidly moving organisms. There should be no gaps under the edges of the frame that would allow for washing of water under the net and loss of benthic organisms. Remove the sample after a specified period of time, by inverting the net into the sample container (wide-mouthed jar) with 10% buffered formalin fixative or 70-80% ethanol. Examine the net closely for small organisms clinging to the mesh, and remove them (preferably with forceps) for inclusion in the sample. Standard method 10500 describe similar collection methods (APHA, 1999).	
Data Uses/Application	Stream-net samplers collect relatively quantitative and qualitative macroinvertebrate samples from the water column of flowing streams and rivers. The macroinvertebrates collected in the nets will be taxonomically identified and counted to determine the macroinvertebrate composition and abundance in that reach of the river. Composition and abundance data are used to measure the status and trends of environmental pollution and its effects on freshwater, estuarine, and marine macroinvertebrates, to assess water quality criteria, and to monitor surface water quality.	
Advantages	Rapid, reproducible and inexpensive sampling technique for in-stream fauna.	
Limitations	It is difficult to set in some substrate types, such as large rubble. It cannot be used effectively in still, slow moving streams. Organisms often wash under the bottom edge of some samplers such as the Surber.	
Reference	USEPA. 1990b. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleORD.html	Last Accessed: 2/13/03

Fact Sheet No.	2.3.1-18	
Method Title	Mussel Collection Using Brails	
Purpose	To sample bivalve mussels in large (non-wadeable) rivers	
Method Summary	A crowfoot brail will be dragged a measured distance of 100 meters. Each brail sample is then brought on board the boat, sorted and counted. The area sampled is calculated in square meters by multiplying the length of the brail by 100 m. Catch success is expressed in terms of the average catch of mussels per square per drag. Brail sampling is randomized within fishing areas and by time periods during two complete harvest seasons. The crowfoot brails can often be made or rented from a commercial fisherman. A minimum of six 100 m long hauls (drags) should be accomplished where a single brail is used. If a significant mussel population is found, then qualitative or quantitative SCUBA samples should be taken. All samples should be identified to species, growth cessation rings counted, and measured for determination of population age structure.	
Data Uses/Application	Brail sampling provides both qualitative and quantitative data pertaining to mussel abundance in a given region. Useful in estuarine areas where mussels comprise a dominant benthic community.	
Advantages	Brail sampling is an inexpensive, bioassessment technique for those riverine environments where mussels are an important component of the ecosystem.	
Limitations	Mussel fishing with brails is highly dependent on experience of the user; however, they are very efficient in the hands of experienced users as attested to by almost 100 years of continuous use.	
Reference	USEPA. 1990b. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/clariton/clhtml/pubtitleORD.html	Last Accessed: 2/13/03

Fact Sheet No.	2.3.1-19	
Method Title	Electrofishing	
Purpose	Fish collection method for environmental assessments	
Method Summary	Electrofishing is a method for collecting fish using electricity. Most electrofishing in freshwater is done with pulsed DC electrical current equipment. In a boat-rigged shocker (boom shocker), one or two people net the fish and another operates the boat and equipment. The fish are nearly always driven into cover as a result of electric stimulus making them difficult to capture. Once driven from cover, the fish are kept within effective range of the electrical field and are immobilized making it possible to pick them up with long-handled dip nets. Other USEPA documents and APHA Standard Method describe similar use of electrofishing for sample collection methods (Barbour et al, 1999; USEPA, 1998; APHA, 1999).	
Data Uses/Application	Electrofishing is a technique used to survey the biological community. As a result, fish species will be identified and counted to determine the organism abundance and composition in that region. Abundance and composition data are used to measure the status and trends of environmental pollution and its effects on marine, estuarine and freshwater organisms, to assess water quality criteria, and to monitor surface water quality.	
Advantages	Efficient method that can be used to obtain reliable information on fish abundance, length-weight relationships, and age and growth of fish in most streams of order 6 or less. Usually results in more consistent success under varying conditions than ordinary seining. It allows greater standardization of catch per unit effort, it requires less time and manpower than use of ichthyocides, and it is less selective than seining (although it is selective towards size and species). If properly used, adverse effects on fish are minimized, and it is appropriate in a variety of habitats.	
Limitations	Individuals involved in electrofishing must have completed a certified course in electrofishing or have been trained by someone certified and experienced in electrofishing. If target assemblage is a common species, then seining may be just as effective. Cannot be used in water with high turbidity. Need very specific conditions and equipment. Sampling efficiency is affected by turbidity, conductivity, aquatic vegetation, depth etc; although it is less selective than seining, electrofishing also is size and species specific. Effects of elctrofishing increase with body size. Species specific behavioral and anatomical differences also determine vulnerability to electroshocking. Electrofishing is a hazardous operation that can injure field personnel.	
Reference	USEPA. 1993c. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA/600/R-92-111. Office of Research and Development, Washington, D.C.	
Website	http://www.epa.gov/bioindicators/html/fish_methods.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-20	
Method Title	Chemical Fishing	
Purpose	Fish collection method for environmental assessments	
Method Summary	Fish toxicants are used for sampling fish populations in impounded waters and streams throughout the United States. Only registered fish chemical toxicants, such as rotenone, cresol, copper sulfate, antimycin A and sodium cyanide, can be used to collect fish in the U.S. The ideal icthyocide is nonselective; easily, rapidly, and safely used; readily detoxified; and not detected and avoided by fish. Chemical sampling is usually employed on a spot basis (*e.g.*, a short reach of river or an embayment of a lake or reservoir). A concentration of 0.5 ppm active ingredient will provide good recovery of most species of fish in acidic or slightly alkaline water. Emulsion products are applied via manual pumps , spraying equipment, power-driven pumps, or a drip spout coming from a flowing system. Standard Method 10600 describes similar uses of chemical fishing for fish sample collection (APHA, 1999).	
Data Uses/Application	Chemical fishing surveys are often used to gain information on particular species of fish and an overall estimation of fish populations and communities. Fish population and community data are used to measure the status and trends of environmental pollution and its effects on freshwater, estuarine and marine organisms, to assess water quality criteria, and to monitor surface water quality. Rotenoning provides greater standardization of unit of effort than seining. Rotenoning has the potential, if used effectively, to provide more complete censuring of the fish population than seining or electrofishing.	
Advantages	Advantages of rotenone: The effective use of rotenone is independent of habitat complexity.	
Limitations	Disadvantages of rotenone: Use of rotenone is prohibited in many states, application and detoxification can be time and manpower intensive. Effective use of rotenone is affected by temperature, light, dissolved oxygen, alkalinity, and turbidity. Rotenoning typically has a high environmental impact; concentration miscalculations can produce substantial fish kills downstream of the study site.	
Reference	USEPA. 1993c. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA/600/R-92-111. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/bioindicators/html/fish_methods.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-21	
Method Title	Fish Collection Using Seine Nets	
Purpose	Fish collection method for environmental assessments	
Method Summary	A strip of strong netting is hung between a stout float line and a strong, heavily weighted lead line at the bottom. In deepwater, one end of the hauling lines is anchored on shore and the boat plays out the line until it reaches the end. The boat then lays out the net parallel to the beach. When all of the net is in the water, the boat brings the end of the second hauling line ashore. The net is then beached as rapidly as possible. In shallow waters, a person can lay out the one end of the hauling line and replicate the role of the boat. There are many different types of seines; selecting the appropriate seine depends on the study design, sampling methods and habitat type. Other USEPA documents and APHA Standard Methods describe the similar use of seine nets for fish collection (Barbour et al, 1999; USEPA, 1998; Standard Method 10600, APHA, 1999).	
Data Uses/Application	Seine nets survey the biological community by collecting abundance and taxa composition data from sampling locations. Organism abundance and composition data are used to measure the status and trends of environmental pollution and its effects on freshwater, estuarine and marine organisms, to assess water quality criteria, and to monitor surface water quality. The Rapid Bioassessment Protocols (RBPs) list seining as a viable way to collect fish samples.	
Advantages	Seines are lightweight and easily transported and stored. Seine repair and maintenance are minimal and can be accomplished onsite. Seine use is not restricted by water quality parameters. Effects on the fish population are minimal because fish are collected alive and are generally unharmed.	
Limitations	Not effective in deep water. Not effective in areas that have snags, large rocks and boulders, or sunken debris that may tear or foul the net. Quantitative seining is very difficult. Previous experience and skill, knowledge of fish habitats and behavior and sampling effort are probably more important in seining than in the use of any other approaches. Seining sample effort and results are more variable than sampling with electrofishing and rotenoning. Seine use is generally restricted to slower water with smooth bottoms, and is most effective in small streams or pools without litter cover or debris. Standardization of unit of effort to ensure data comparability is difficult	
Reference	USEPA. 1993c. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA/600/R-92-111. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/bioindicators/html/fish_methods.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-22	
Method Title	Entanglement Nets	
Purpose	Fish collection method for environmental assessments	
Method Summary	Entanglement nets, including gill nets and trammel nets, are used to sample fish populations in estuaries, lakes, reservoirs, and larger rivers. Gill nets are the more commonly used entanglement nets. They are usually set as an upright, vertical fence of netting and can have either a variable or uniform mesh size. Gill nets selectively capture particular species of fish since the mesh size determines the size range of the fish to be sampled. They can be set at the surface, mid-depth, or on the bottom depending on the objectives of the study and target species within the fish community. Trammel nets are used in all types of riverine habitat. If a river channel is to be fished, the net is floated or drifted downstream. They are very efficient for taking fish like carp, buffalo, shovelnose sturgeon and freshwater drum.	
Data Uses/Application	Entanglement nets survey the biological community by collecting abundance and taxa composition data from sampling locations. They are often used to gain information on particular species of fish and an overall estimation of fish populations and communities. Fish population and community data are used to measure the status and trends of environmental pollution and its effects on freshwater, estuarine and marine organisms, to assess water quality criteria, and to monitor surface water quality.	
Advantages	This method describes an effective way to sample fish populations. The results are expressed as the number or weight of fish taken per length of net per day (catch per unit effort).	
Limitations	Entanglement nets need to be monitored for by-catch. Non-target species may be caught and will not survive long in the net. Tidal currents, predation, optimum fishing time and types of anchors, floats and line must be considered when setting entanglement nets in estuaries.	
Reference	USEPA. 1993c. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA/600/R-92-111. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/bioindicators/html/fish_methods.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-23	
Method Title	Entrapment Devices	
Purpose	Fish collection method for environmental assessments	
Method Summary	With entrapment devices, fish enter an enclosed area (which may be baited) through a series of one or more funnels. They are set in structurally complex areas where fish movement and density are anticipated to be highest in order to maximize net catches. Common entrapment devices include the hoop net, fyke net, and minnow trap.	
Data Uses/Application	Entrapment devices survey the biological community by collection abundance and taxa composition data from sampling locations. They are often used to gain information on particular species of fish and an overall estimation of fish populations and communities. Fish population and community data are used to measure the status and trends of environmental pollution and its effects on freshwater, estuarine and marine organisms, to assess water quality criteria, and to monitor surface water quality.	
Advantages	They are used to sample reservoirs and wide river channels with slow velocity conditions. The catch is recorded as numbers of weight per unit of effort, usually fish per net day.	
Limitations	Entrapment devices are generally deployed overnight, requiring separate filed activity for deployment and retrieval. Traps are often vandalized in unsecured areas.	
Reference	USEPA. 1993c. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA/600/R-92-111. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/bioindicators/html/fish_methods.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-24	
Method Title	Pop Nets	
Purpose	Fish collection method for environmental assessments	
Method Summary	Pop nets are rectangular devices, constructed of mesh netting used for the collection of fish. They are designed to be deployed from the surface and released with a mechanical device. They are set and received by two individuals and are easily dissembled for transport.	
Data Uses/Application	Pop nets survey the biological community by collecting abundance and taxa composition data from sampling locations. They are often used to gain information on particular species of fish and an overall estimation of fish populations and communities. Fish population and community data are used to measure the status and trends of environmental pollution and its effects on freshwater, estuarine and marine organisms, to assess water quality criteria, and to monitor surface water quality.	
Advantages	Useful for sampling fish in shallow, riverine waters in heavily vegetated areas and nonvegetated areas where seining or electroshocking may be difficult.	
Limitations	Pop nets will sample a relatively small area/volume of surface water. Thus, representativeness may be a concern if used for population studies.	
Reference	USEPA. 1993c. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/R-92-111. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/bioindicators/html/fish_methods.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-25	
Method Title	Trawls	
Purpose	Fish collection method for environmental assessments	
Method Summary	Trawls are designed like seines; however, they are much larger and intended to be towed behind boats in large, open water areas. There are four types of trawls available: beam trawls, used to capture bottom fish; otter trawls, used to capture near-bottom and bottom fish; mid-water trawls, used to collect schooling fish at various depths; and surface tow nets, used to collect fish at or near the surface. The trawls are deployed behind the boat, often with power winches and large motors. Under the Coastal EMAP Program, fish are specifically collected with a high rise sampling trawl with a 13.5 meter footrope with a chain sweep. Tow duration is 10 minutes with a towing speed of 2-3 knots against the prevailing current. Speed over the bottom should be 1-3 knots (USEPA, 2000b). The trawl is retrieved used hydraulics and the contents of the net are often emptied onto sorting tables. Fish are sorted, enumerated and examined for gross pathological examinations. Selected specimens are retained and properly processed for tissue chemical analyses. The APHA Standard Method describe similar trawl methods to collect fish (Standard Method 10600 (APHA, 1999).	
Data Uses/Application	Trawl nets survey the biological community by collecting samples that will be analyzed for species composition, relative abundance, chemical analysis, and pathological examination. They are often used to gain information on particular species of fish and an overall estimation of fish populations and communities. Fish population and community data are used to measure the status and trends of environmental pollution and its effects on freshwater, estuarine and marine organisms to assess water quality criteria, and to monitor surface water quality.	
Advantages	Trawl nets are very effective in large, open water areas and can effectively sample selected bottom, mid-water, and surface oriented species at specific life history stages.	
Limitations	Not effective in deep water. Not effective in areas that have snags, large rocks and boulders and sunken debris that may tear or foul the net. Quantitative trawling is very difficult. Previous experience and skill, knowledge of fish habitats and behavior and sampling effort are probably more important in seining than in the use of any other approaches. Trawling sample effort and results are more variable than sampling with electrofishing and rotenoning. Trawl use is generally restricted to slower water with smooth bottoms, and is most effective in small streams or pools without litter cover or debris. Standardization of unit of effort to ensure data comparability is difficult	
Reference	USEPA. 1993c. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/R-92-111. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/bioindicators/html/fish_methods.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-26	
Method Title	Fish Processing Method in the Great Lakes, LMMB 025	
Purpose	This method describes the aging, compositing, and grinding method for fish.	
Method Summary	Whole fish are collected (intact, with all body fluids and no incisions), wrapped in aluminum foil, placed in 4 mil thick polyethylene bags, tagged, and frozen as soon as possible on board the vessel. Fish should be transferred in coolers and stored at -20°C. To age the fish, scales were removed from the fish, and the annual rings on the scales are read to determine the age. Some fish that have been stocked may contain coded wire tags (CWT) or clipped fins. Stocking or tagging records may provide useful information in aging such fish. For homogenization, fish are removed from the freezer and allowed to thaw in their bags over an 8 - 12 hour period. The contents of the bags are weighed and recorded. Fish may be composited based on species, location, size and season sampled. Fish are measured (millimeters) on a measuring board and weighed to the nearest gram. The measuring board, scalpel, and balance are cleaned between each group. Each composite sample is homogenized, using various size vertical cutters or Robot Coupe cutter. Subsamples of the homogenized tissue are placed in clean sample containers and frozen (-20°C) until analyzed. Standard Method 10600D.3 also discusses the field processing of fish, including length, weight, and age measurements (APHA, 1999).	
Data Uses/Application	Fish are often collected for chemical analyses of tissue to determine whether contaminants are accumulating in biological populations.	
Advantages	These are standard methods and are performed fairly consistently to prepare tissue for chemical analyses which enhances data consistency in resultant data.	
Limitations	Study design should consider advantages/limitations of homogenates of whole body, fillet, or offal.	
Reference	USEPA. 1997b. Method LMMB 025: Fish Processing Method. Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, Chicago, IL.	
Website	http://www.epa.gov/glnpo/lmmb/methods/fpmlmmb.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-27
Method Title	Fish Processing
Purpose	To determine fish diversity and abundance
Method Summary	Once a catch is brought on deck, fish are identified to species, measured, counted, examined for external pathology and processed for chemical analysis. The fish are measured with a measuring board, the fork length to the nearest millimeter. Coastal EMAP procedures specify that the first 30-50 individuals of each species should be measured. The remaining fish will be identified to species and counted (USEPA, 2000b, EPA/620/R-00/002). All individuals that are measured, will be examined for evidence of gross external pathology. The examination is intended to be a rapid scan of the surface of individual. Fish should be cut the entire length of the abdominal cavity. The later musculature is removed from one side of the animal's visceral cavity to facilitate the fixation of the internal organs. The opercula is removed and immersed in fixative. The sample (whole fish or head, visceral cavity and abnormalities excised) is placed in a plastic bag with many perforations. This bag is then placed in the fixative. Specimen's should be fixed in Dietrich's fixative for one or two days . Samples may be transferred to another preservative, such as ethyl alcohol (70-75%) or isopropanol (40-45%), for storage. Samples for fish tissue contaminant analysis or electrophoresis must be iced, placed in dry ice, or liquid N_2 for temporary storage or shipping. Special preservation techniques must be used for histological, histochemical, or biomarker analyses. Samples are then identified to the species level. Data recorded include species composition and diversity, population density and biomass, and physiological condition of indigenous communities of aquatic organisms.
Data Uses/Application	Species relative abundance and taxa richness are data derived from these protocols. These data parameters provide information pertaining to the status and trends of environmental pollution and its impacts on freshwater, marine and estuarine communities. Biological impairment resulting from pollution is often evaluated using metrics of biotic integrity derived from the aforementioned data parameters that evaluate community, population and functional parameters. Fish metrics commonly determined from this data include species richness, trophic composition, and fish abundance and condition. Metrices are collectively evaluated in indices such as the Index of Biotic Integrity (IBI) which aggregates 12 biological metrics to assess fish assemblage data.
Advantages	These are considered basic and standard methods for fish processing.

Fact Sheet No.	2.3.1-27 (contd.)	
Limitations	As with any tissue processing, care must be taken to avoid laboratory and cross-contamination.	
Reference	Barbour *et al.* 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition, EPA 841-B-99-002. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/owow/monitoring/rbp/	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.1-28	
Method Title	Swallows: Sampling Procedures	
Purpose	This method describes the use of tree swallows at contaminated sites to quantify population level impacts and population level chemistry data.	
Method Summary	Approximately 60 swallow boxes, 30 at each of 2 sites, will be attached to posts or other suitable structures in suitable habitat. Boxes will be placed approximately 20-30 meters apart, but this can vary depending on the structure of the habitat. Each nest box will be visited approximately once per week until egg laying begins. After that time, nests may be visited more often to collect egg or just hatched young samples. After the eggs have been hatched, boxes will be visited at least once per week until the young reach 12 days of age. Whether eggs or young are present in the nest box, the number of eggs and young present will be recorded on a data sheet. A sample of 2-3 eggs and/or just-hatched eggs (pippers) and a sibling 12-day-old tree swallow nestling will be collected from a minimum of 5-10 boxes at each site. Food samples from the stomachs of tree swallow nestlings will be removed at the time that they are collected and dissected. A pooled food sample from each site, along with the pipper and nestling samples may be analyzed for organochlorine chemicals, including total PCBs and a full dioxin scan if sufficient mass is available. Nestling tree swallows may be ligatured to obtain additional food samples for insect species identification and for chemical analysis of food. Ligatures, black electrical zip ties, will be placed on all nestlings in a nest box and left in place for 1 hour. Care will be taken that the zip ties are loose enough to allow normal breathing. After 1 hour, the food boli will be removed from the throats of the nestlings using a pair of blunt-nosed forceps and the ligatures removed.	
Data Uses/Application	Swallows are collected for determining population impacts (*i.e.,* reproductive successes, deformities etc) and for chemical analyses.	
Advantages	Evaluation of population level impacts is important to determine potential exposures and effects over the long term. Chemical analyses of swallow tissue indicate whether or not bioaccumulative chemicals are present and whether they are being transferred up the food chain.	
Limitations	Sampling bird species is less precise since their mobility allows them to forage in a great range of areas. Therefore, population level and tissue contaminant level results may not always be indicative of the conditions at the site of concern. Single species tests sometimes skew results since species will react differently to contaminant exposures due to various biochemical or physiological traits.	
Reference	USGS. 1998. Tree Swallow Sample Collection and Processing Procedures, Technical Operating Procedure WE-410.0. Upper Mississippi Science Center, U.S. Geological Survey, LaCrosse, WI.	
Website	N/A	Last Accessed:

Fact Sheet No.	2.3.1-29	
Method Title	Sample Processing of Swallows	
Purpose	This method describes the use of tree swallows at contaminated sites to quantify population level impacts and population level chemistry data.	
Method Summary	Pippers or 12-day old nestlings will be removed from the nest box and weighed. Pippers and nestlings will be visually examined for gross abnormalities. Nestlings will be decapitated with a pair of sharp scissors, contents in the upper gastrointestinal tract removed with forceps after an incision is made along the length of the stomach, and the carcass remainder placed in a chemically clean jar, which has been purchased in that condition. The above will be done within 2 hours after removal from the nest box. The carcass remainders and food samples will be maintained frozen until transported to the storage in a freezer. Samples will be shipped to an analytical laboratory for processing.	
Data Uses/Application	Swallows are collected for determining population impacts (*i.e.,* reproductive successes, deformities etc) and for chemical analyses used in ecological risk assessments. Evaluation of population level impacts is important to determine potential exposures and effects over the long term. Chemical analyses of swallow tissue indicate whether or not bioaccumulativve chemicals are present and whether they are being transferred up the food chain.	
Advantages	Swallows are often used in ecological risk assessments at freshwater ponds because of their exclusive insect diet and comparative ease of collection.	
Limitations	Sampling bird species is less precise since their mobility allows them to forage in a great range of areas. Therefore, population level and tissue contaminant level results may not always be indicative of the conditions at the site of concern. Single species tests sometimes skew results since species will react differently to contaminant exposures due to various biochemical or physiological traits.	
Reference	Custer, C.M., T.W. Custer, P.D. Allen, K.L. Stromborg, and M.J. Melancon. 1998. Reproduction and environmental contamination in tree swallows nesting in the Fox River drainage in Green Bay, Wisconsin, USA. Environmental Toxicology and Chemistry. 17:1786-1798.	
Website	N/A	Last Accessed:

2.3.2 Chemical and Physical Analysis

Section 2.3.2 contains methods for sample preparation and the chemical analysis of biota. These methods characterize the chemical composition of biota samples collected by methods described in Section 2.3.1. Samples are often analyzed for the presence of inorganic and organic contaminants that may pose a threat to human or ecological health. Analyzing biological tissue provides a direct measure of the uptake and bioaccumulation of pollutants from the environment and can be used to correlate environmental concentrations of contaminants with body residues.

Many of the methods described have been developed over time to optimize the detection, identification, and quantification of potential chemicals of concern. Several are performance-based and may be further modified to enhance the accuracy and precision of the method. Special preparation and clean up procedures are utilized when analyzing biological tissue, due to the often high lipid content of the samples. Lipids tend to concentrate environmental pollutants, but they can also interfere with the analysis of these contaminants.

The chemical methods for the analysis of biological tissue are less routinely performed than for the analysis of water and sediment. NOAA's National Status and Trends Program (1998) developed many methods for the chemical analysis of biota and was a main source of information in the preparation this compendium. Other sources of information presented in Section 2.3.2 included:

- The USEPA's Office of Water
- The USEPA's Lake Michigan Mass Balance Study Methods Compendium, 1997v
- The USEPA's Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, 1993
- The USEPA's Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (SW846 Methods)
- Standard Methods for Examination of Water and Wastewater, 1999
- ASTM

Fact Sheet No.	2.3.2-1	
Method Title	Sample Preparation for Metal Contaminants in Tissue	
Purpose	This method describes how fish tissue is processed and prepared for metal contaminant analyses in the laboratory.	
Method Summary	Prior to use, utensils and bottles should be thoroughly cleaned with a detergent-free solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. Sample size requirements vary with tissue type and detection limit requirements. When filleting the fish, special care should be taken to avoid contaminating target tissues (especially muscle) with slime and/or adhering sediment from the fish skin. The procedure previously outlined for the preparation of fillet samples should generally be followed. Unless specifically sought as a sample, the dark muscle tissue that may exist in the vicinity should not be separated from the light muscle tissue. Samples should be frozen after resection and kept at -20°C. Samples may or may not be homogenized before acid digestion and subsequent preparation before analysis. ASTM Method D4309 also describes the preparation of biological samples for inorganic chemical analysis (ASTM, 2001a).	
Data Uses/Application	This method is followed by investigators preparing fish tissue for trace metal analysis.	
Advantages	Control of metal contamination is addressed in this method.	
Limitations	The major difficulty in trace metal analyses of tissue samples is controlling contamination of the sample after collection. In the field, sources of contamination include sampling gear, grease from winches or cables, engine exhaust, dust or ice used for cooling. Sample resection and any subsampling of the organisms should be carried out in a controlled environment (*i.e.*, a Class 100 clean room).	
Reference	USEPA. 1993c. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA-600-R-92-111. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/bioindicators/html/fish_methods.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-2	
Method Title	Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation, Appendix to Method 1631	
Purpose	These procedures may be used in conjunction with EPA Method 1631B for determination of mercury in tissue, sludge, sediment, soil, industrial samples, and certified reference materials.	
Method Summary	Digestion I—This procedure is preferred for matrices containing organic materials, such as sludge and plant and animal tissues, because the organic matter is completely destroyed. In this procedure, a 0.2 - 1.5 g sample is digested with HNO_3/H_2SO_4. The digestate is diluted with BrCl solution to destroy the remaining organic material. Digestion II—This procedure is preferred for geological materials because of rapid and complete dissolution of cinnabar (HgS), which is otherwise more slowly attacked by the BrCl in Digestion I. In this procedure, a 0.5 - 1.5 g sample is digested with aqua regia (HCl/HNO_3) to solubilize inorganic materials. The Hg concentration in the digestate is determined using EPA Method 1631B. These procedures, in conjunction with Method 1631B, allow determination of Hg at concentrations ranging from 1.0 to 5000 ng/g in solid and semisolid matrices. The method detection limit for Hg has been determined to be in the range of 0.24 to 0.48 ng/g when no interferences are present. The minimum level of quantization (ML) has been established as 1.0 ng/g. These levels assume a sample size of 0.5 g. Using Method LMMB 053 (USEPA, 1997d) to measure total mercury in fish, the fish tissue is digested in nitric acid for 30 minutes at room temperature then in a bomb at 190°C for 15 minutes. The digestate is diluted to 25 mL using Milli-Q water and sample aliquots are analyzed using the CVAFS purge and trap method. Specific procedures for the measurement of mercury in plankton are described in Method LMMB 051 (USEPA, 1997d).	
Data Uses/Application	The extent of mercury bioaccumulation is an important parameter to support human health, ecological risk assessments and bioaccumulation models.	
Advantages	The dual amalgam trap system and fluorescence detector provide greater sensitivity and specificity in the presence of interferences, and this system must be used to overcome interferences, if present, and to achieve the sensitivity required, if necessary.	
Limitations	This method does not measure methyl mercury, which may need to be monitored separately in contaminated sediments.	
Reference	USEPA. 2001d. Appendix to Method 1631: Total Mercury in Sludge, Sediment, Soil, and Tissue by Acid Digestion and BrCl Oxidation, EPA-821-R-01-013. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.brooksrand.com/FileLib/1631e.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-3	
Method Title	Versatile Combustion-Amalgamation Technique for the Photometric Determination of Mercury in Fish and Environmental Samples, LMMB 052	
Purpose	For the direct detection of total mercury in fish and environmental samples.	
Method Summary	0.05 - 0.1 g fish tissue is heated in a stream of O_2 for 3.5 minutes in an induction furnace. The released mercury vapor passes through a series of traps, and the mercury is collected in a 10 mm diameter column of 24-gauge gold wires. This amalgam is heated in the induction furnace and volatilized mercury is measured with a mercury vapor meter. The detection limit is less than 0.002 µg.	
Data Uses/Application	The extent of mercury bioaccumulation is an important parameter to support human health, ecological risk assessments and bioaccumulation models.	
Advantages	This analytical system is easily converted to handle biological materials, water, and sediments. Total analysis time is about 8 minutes per sample and a single analyst can make up to 40 determinations in eight hours. The method has high sensitivity, precision, and accuracy. Also, a small sample size is required.	
Limitations	For analysis of samples outside the range of 0.02 - 5.0 ppm mercury, a change of operating procedure is required. Frequent changes in attenuation of the mercury vapor meter are required. The sample must be well homogenized, due to the very small sample size analyzed with this method. Detection limits may not be sufficiently low for ecological or human health risk assessments.	
Reference	USEPA. 1997d. Method LMMB 052: Versatile Combustion-Amalgamation Technique for the Photometric Determination of Mercury in Fish and Environmental Samples, Lake Michigan Mass Balance Study Methods Compendium, Volume 2: Organic and Mercury Sample Analysis Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/lmmb/methods/scan.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-4
Method Title	Trace Element Quantification Techniques
Purpose	To determine major and trace elements in sediment and biological tissue samples utilizing atomic absorption and neutron activation techniques.
Method Summary	Tissue samples are homogenized and freeze dried, and a dry aliquot is homogenized and transferred to a Teflon™ bomb. Samples are digested by adding 3 mL HNO_3 and leaving the bombs at room temperature overnight. The bombs are then placed in a 130°C oven for approximately 20 hours. After cooling, 18 mL of quartz distilled water are added, and the solution volume is determined, and a 20-fold dilution is made for FAAS analysis of Al, Fe, Mn, Si, and Zn. For analysis of Hg, sediment samples are digested using a modified version of EPA method 245.6. Samples were analyzed using the following instrumentation: **Analyte** — **Method** Hg — Cold vapor atomic absorption (CVAA) Cu, Fe, Zn — Flame atomic absorption (FAA) Ag, As, Cd, Cr, Cu, Ni, Pb, Se, Sn — Graphite furnace atomic absorption (GFAA) Ar, Cr, Fe, Se, Ag, Zn — Instrumental neutron activation analysis (INAA) ASTM Method D1971 describes the digestion of samples for determination of metals by Flame Atomic Absorption (ASTM, 2001a). Standard Method 3030K describes the microwave digestion method (APHA, 1999). Several Standard Methods describe the analysis of metals using various methods: SM 3112 B for CVAA; SM 3111 for FAA; SM3113B for GFAA.
Data Uses/Application	Methods provide low detection limits needed for measuring ambient concentrations at uncontaminated sites, or reference sites.
Advantages	Tissue sample digestion in a Teflon™ bomb is a standard method for "clean" digestion for metals analysis. The instrumental suite employed in this method takes advantage of the know strengths of each instrument for trace analysis. For example, GFAA is much more sensitive than FAA, requiring only a small volume of sample for trace analysis.
Limitations	Requires multiple instrument analyses for results on a complete suite of elements.
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.
Website	http://ccma.nos.noaa.gov/publications/tm130.pdf — Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-5
Method Title	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Purpose	To determine the concentration of 17 metals in sediment and biological tissue samples utilizing atomic absorption, inductively coupled plasma mass spectrometry (ICP-MS), and energy dispersive X-Ray fluorescence (XRF).
Method Summary	Tissue samples are weighed and freeze-dried. The dried sample is ground in a mill. 0.5 g aliquots are used directly for XRF analysis or are further digested for AA or ICP-MS analysis. 500 mg of dried tissue is placed in a Teflon™ bomb, to which 5 mL of HCl and 3.5 mL of HNO_3 are added. The bombs are heated in a 60°C water bath for 3-4 hours. After cooling, the bombs are heated in a 130°C oven for 16 hours. After cooling, the digestates are diluted to approximately 20 mL with deionized water. Solution volumes are calculated, and the digestates are analyzed directly by GFAA and CVAA or diluted 10:1 for ICP-MS analysis. Analyte — Method Hg — Cold vapor/gold foil amalgam Al, Cd, Cr, Ni, Ag, Pb — Graphite furnace atomic absorption (GFAA) Ag, Al, Cr, Cd, Ni, Pb, Sb, Sn — ICP-MS As, Cu, Fe, Mn, Se, Si, Zn — XRF Several Standard Methods exist for the analysis of metals by a variety of methods: 3112B for CVAA; 3113B for GFAA; 3120B for ICP-MS (APHA, 1999).
Data Uses/Application	Metals contaminant data are used in both ecological and human health risk assessments.
Advantages	XRF analysis does not require digestion of the sample. Se and As, which can be difficult to analyze in tissue by ICP-MS, are easier to analyze by XRF. The digestion solution of HCl/HNO_3 provide better recoveries for Ag. ICP-MS has the advantage of simultaneous analysis of many elements with detection limits much lower than the XRF and similar to those of GFAA. ICP-MS is particularly sensitive for Al, Cr, Ni, Ag, Cd, Sn, Sb, P, and Tl. CVAA is very sensitive and reliable for Hg analysis.
Limitations	Leakage at high pressure can cause loss of Hg from the sample during digestion. Analysis of GFAA requires the use of matrix modifiers and standardization of the instrument by method of addition to the sample matrix to provide accurate results.
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.
Website	http://ccma.nos.noaa.gov/publications/tm130.pdf — Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-6
Method Title	Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry, EPA Method 1632, Revision A
Purpose	This method is for determination of inorganic arsenic (IA), arsenite (As +3), arsenate (As +5), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) in tissue by hydride generation and quartz furnace atomic absorption detection. This method is designed for measurement of As species in tissue in the range 0.10-500 µg/g dry weight.
Method Summary	A 10- to 50-g wet weight sample is collected into a sample bottle. The tissue sample is either freeze-dried and stored at room temperature or stored frozen at less than -18°C. Prior to analysis, tissue samples are digested in HCl or NaOH at 80°C for 16 hours. An aliquot of tissue digestate is placed in a specially designed reaction vessel, and 6M HCl is added. $NaBH_4$ solution is added to convert IA, MMA, and DMA to volatile arsines. Arsines are purged from the sample onto a cooled glass trap packed with 15% OV-3 on Chromosorb ® W AW-DMCS, or equivalent. The trapped arsines are thermally desorbed, in order of increasing boiling points and carried into the quartz furnace of an atomic absorption spectrophotometer for detection. To determine the concentration of As +3, another aliquot of water sample or tissue digestate is placed in the reaction vessel and Tris-buffer is added. The procedure is repeated to quantify only the arsine produced from As +3. The concentration of As +5 is the concentration of As +3 subtracted from the concentration of IA. Analyte — MDL — ML IA (As +3 +As +5) — 0.03µg/g — 0.10 µg/g Arsenite (As +3) — 0.02 µg/g — 0.10 µg/g MMA — 0.01 µg/g — 0.05 µg/g DMA — 0.04 µg/g — 0.10 µg/g
Data Uses/Application	The method is for use site characterizations and risk assessments.
Advantages	The relative amounts of carcinogenic arsenite (As +3) to total arsenic varies with surface water body and varies with pH. This method directly quantifies arsenite.
Limitations	This method is far more costly than total arsenic determination and may only be needed where speciation is required.
Reference	USEPA. 2001c. Method 1632, Revision A: Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry, EPA 821-R-01-006. Office of Water, U.S. Environmental Protection Agency, Washington, DC.
Website	http://www.brooksrand.com/methods/1632a.pdf — Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-7	
Method Title	Extraction and Lipid Separation of Fish Samples for Contaminant Analysis and Lipid Determination, LMMB 043	
Purpose	To prepare tissue samples for the measurement of organic contaminants, such as polychlorinated biphenyls, polynuclear aromatic hydrocarbons, and chlorinated pesticides.	
Method Summary	Using Method LMMB 043 (USEPA, 1997d), 10 g of tissue are combined with sodium sulfate. The tissue mixture is transferred to a rinsed chromatography column that is plugged with glass wool. The tissue is extracted twice by eluting the column with 50 mL of a 90/10 petroleum ether/ethyl acetate mixture. The elutant is concentrated using a Turbovap. A GPC column is used to remove lipids from the extract. Samples are solvent exchanged into iso-octane and cleaned up using a silica gel column. Extracts are eluted using a 5/95 ethyl acetate/hexane solvent mixture. Samples are concentrated for analysis. Using NS&T procedures (NOAA, 1998), a 0.5 - 15 gram (wet weight) aliquot of the homogenized tissue sample is spiked with surrogate standards and extracted three times with dichloromethane in the presence of sodium sulfate by maceration with a Tissumizer™. The extract is filtered through glass wool and sodium sulfate after each extraction. The extract is concentrated using the Kuderna-Danish technique and solvent changed to hexane. The sample is cleaned-up using alumina/silica gel column chromatography before instrumental analysis. Tissue samples require further purification by gel permeation chromatography (GPC) prior to instrumental analysis for pesticides and PCBs. The sample is concentrated to 1 mL in hexane for analysis. US EPA's Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters (USEPA, 1993c) also describe sample preparation methods for organic contaminants in tissue.	
Data Uses/Application	Tissue contaminant data are used to delineate the spatial and temporal extent of contamination and used in ecological and human health risk assessments.	
Advantages	This method provides quantitative extraction of most organic contaminants from tissue samples, including those with high lipid contents.	
Limitations	These extraction methods have been validated for non-polar persistent organic contaminants, such as PCBs, chlorinated pesticides, and PAHs. Methods may not be applicable for more polar compounds or more reactive compounds.	
Reference	USEPA. 1997d. Method LMMB 043: Extraction and Lipid Separation of Fish Samples for Contaminant Analysis and Lipid Determination, Lake Michigan Mass Balance Study Methods Compendium, Volume 2: Organic and Mercury Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/lmmb/methods/hc521a.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-8	
Method Title	Purification of Biological Tissue Samples by Gel Permeation Chromatography of Organic Analyses	
Purpose	To purify tissue extract samples by separating out lipids and high molecular weight components from target compounds.	
Method Summary	The GPC/HPLC is calibrated to verify the instrument performance based on retention time and area of each of the calibration standards. Sample extracts are processed through a guard column and two size exclusion columns connected in series and the desired fraction is collected with a fraction collector. The collected fraction is then concentrated and analyzed for polycyclic aromatic hydrocarbons, pesticides, and polychlorinated biphenyls.	
Data Uses/Application	This method is a clean-up step used in the processing of organic contaminant sample extracts for GC/MS or GC/ECD analysis.	
Advantages	A large amount of neutral lipids and high molecular weight components from tissue samples can be eluded from an alumina/silica gel column chromatography clean up step. Size exclusion chromatography can separate the target analytes from these other components. Upon calibration, this method is also suitable for the isolation of other classes or organic contaminants.	
Limitations	This method isolates organic contaminants from lipid matrix but does not separate or isolate individual fractions of organic contaminants.	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	http://ccma.nos.noaa.gov/publications/tm130.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-9	
Method Title	Quantitative Determination of Polynuclear Aromatic Hydrocarbons by Gas Chromatography/Mass Spectrometry (GC/MS) - Selected Ion Monitoring (SIM) Mode	
Purpose	To determine low concentrations of polycyclic aromatic hydrocarbons (PAHs) and their alkylated homologues in extracts of water, sediments and biological tissues.	
Method Summary	Just prior to analysis, an aliquot of internal standard solution is added to the sample extract producing a final internal standard concentration of approximately 40 ng/mL. The analytical system includes a temperature programmable gas chromatography with a fused silica capillary column. Helium is used as the carrier gas, and the samples are handled by an auto sampler capable of making 1 - 4 µl injections. A five point calibration curve is established to demonstrate the linear range of the detector. The effluent from the GC capillary column is routed directly into the ion source of the mass spectrometer (MS). The MS is operated in the SIM mode using appropriate windows to include the quantization and confirmation masses for target PAHs. For all compounds detected at a concentration above the MDL, a confirmation ion is checked to confirm its presence. The response factors of the surrogate relative to each of the calibration standards are calculated, followed by the calculation of the sample extract concentration. The sample concentration for each compound is calculated by dividing the sample extract concentration by the sample amount.	
Data Uses/Application	PAH data obtained from this analysis are used for site characterization and site assessment.	
Advantages	GC/MS in the SIM mode provides unambiguous and sensitive detection for PAHs. The PAH quantization method is very rigorous because PAHs have very strong molecular ion peaks under the mass spectrometric conditions used. Also, the availability of labeled surrogates internal standards of many of the analytes makes very accurate determinations of analyte concentrations possible. Analysis of alkylated PAH homologues can provide site-specific information that can be used in source identification or product identification.	
Limitations	GC/MS in SIM mode cannot be used for simultaneous screening for other organic contaminants of similar polarity or volatility and cannot be used to identify tentatively identified compounds (TICs).	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	http://ccma.nos.noaa.gov/publications/tm130.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-10	
Method Title	Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection, LMMB 041	
Purpose	To quantify chlorinated hydrocarbons (*i.e.,* chlorinated pesticides and PCBs) in sample extracts.	
Method Summary	This method is based on high resolution, capillary gas chromatography using electron capture detection (GC/ECD). Extracts normally have a holding time of 40 days. The instrument's detector is calibrated before the sample in injected. Pesticide/PCB calibration is done also as part of the analytical run. If the response for any peak exceeds the highest calibration solution, the extract is diluted, a known amount of surrogate and TCMX solution added, and the sample reanalyzed for those analytes that exceeded the calibration range. Concentrations in the samples are calculated based on the internal standard method. Data is reported as ng/g dry weight. Other methods describing the analysis of PCBs and pesticides by GC/ECD are NS&T methods, ASTM Methods D5317 and D3534, and SW846 Methods 8081A and 8082 (NOAA, 1998; ASTM, 2001c).	
Data Uses/Application	Data are used in site characterization and in risk analysis.	
Advantages	The ECD is very sensitive and allows for detection of the chlorinated hydrocarbons at trace concentrations (ppb).	
Limitations	The detector does not have a linear response over a wide concentration range and must be used by sufficiently trained personnel. Second column analysis must be performed to provide unequivocal compound identification. These methods do not measure the 12 World Health Organization congeners, which may be desired data in some risk assessments.	
Reference	USEPA. 1997d. Method LMMB 041: Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection, Lake Michigan Mass Balance Study Methods Compendium, Volume 2: Organic and Mercury Sample Analysis Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.	
Website	http://www.epa.gov/glnpo/lmmb/methods/sop-501.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-11
Method Title	Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, EPA Method 1613
Purpose	This method is for determination of tetra- through octa-chlorinated dibenzo-p-dioxins (CDDs) and dibenzo furans (CDFs) in tissue.
Method Summary	This method is "performance-based." The sample is extracted by one of two procedures: 1.Soxhlet or SDS extraction—A 20 g aliquot of sample is homogenized, and a 10 g aliquot is spiked with the labeled compounds. The sample is mixed with sodium sulfate, allowed to dry for 12-24 hours, and extracted for 18-24 hours using methylene chloride:hexane (1:1) in a Soxhlet extractor. The extract is evaporated to dryness, and the lipid content is determined. 2.HCl digestion—A 20 g aliquot is homogenized, and a 10 g aliquot is placed in a bottle and spiked with the labeled compounds. After equilibration, 200 mL of hydrochloric acid and 200 mL of methylene chloride:hexane (1:1) are added, and the bottle is agitated for 12-24 hours. The extract is evaporated to dryness, and the lipid content is determined. After extraction, $^{37}Cl_4$-labeled 2,3,7,8-TCDD is added to each extract to measure the efficiency of the cleanup process. Sample cleanups may include back-extraction with acid and/or base, and gel permeation, alumina, silica gel, Florisil and activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of the 2,3,7,8-isomers or other specific isomers or congeners. After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, internal standards are added to each extract, and an aliquot of the extract is injected into the instrument. The analytes are separated by the GC and detected by a high-resolution (≥10,000) mass spectrometer. **CDD/CDF** — **ML (pg/µL)** — **CDD/CDF** — **ML(pg/µL)** 2,3,7,8-TCDF — 0.5 — 1,2,3,4,7,8-HxCDD — 2.5 2,3,7,8-TCDD — 0.5 — 1,2,3,6,7,8-HxCDD — 2.5 1,2,3,7,8-PeCDF — 2.5 — 1,2,3,7,8,9-HxCDD — 2.5 2,3,4,7,8-PeCDF — 2.5 — 1,2,3,4,6,7,8-HpCDF — 2.5 1,2,3,7,8-PeCDD — 2.5 — 1,2,3,4,7,8,9-HpCDF — 2.5 1,2,3,4,7,8-HxCDF — 2.5 — 1,2,3,4,6,7,8-HpCDD — 2.5 1,2,3,6,7,8-HxCDF — 2.5 — OCDF — 5.0 1,2,3,7,8,9-HxCDF — 2.5 — OCDD — 5.0 2,3,4,6,7,8-HxCDF — 2.5 This method is also described in SW846 Method 8290
Data Uses/Application	The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.
Advantages	Method 1613 is able to meet detection limits required for human health and ecological risk assessments.
Limitations	The GC/MS portions of this method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons.

Fact Sheet No.	2.3.2-11 (contd.)	
Reference	USEPA. 1994c. Method 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, EPA 821-B-94-005. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/waterscience/methods/1613.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-12
Method Title	Toxic Polychlorinated Biphenyls by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry, EPA Method 1668
Purpose	This method is for determination of the toxic polychlorinated biphenyls (PCBs) in solids (not tissue).
Method Summary	This method is performance-based. A 20-g aliquot of sample is homogenized, and a 10-g aliquot is spiked with the labeled compounds. The sample is mixed with sodium sulfate, allowed to dry for 12- 24 hours, and extracted for 18-24 hours using methylene chloride: n-hexane (1:1) in a Soxhlet extractor. The extract is evaporated to dryness, and the lipid content is determined. After extraction, samples are cleaned up using back-extraction with sulfuric acid and/or base, and gel permeation, silica gel, Florisil and activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of specific isomers or congeners. After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, internal standards are added to each extract, and an aliquot of the extract is injected into the gas chromatography. The analytes are separated by the GC and detected by a high-resolution (≥10,000) mass spectrometer.

IUPAC	**EMDL (ng/kg)**	**EML (ng/kg)**	**Extract EML (pg/µL)**
77	0.5	2	1
123	4	10	5
126	10	4	5
118/167/156/157/169/180/170/189	6	20	10
114	60	200	100
105	40	100	50

EMD: = Estimated Method Detection Limit; EML = Estimated Minimum Level

Data Uses/Application	The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.
Advantages	Method 1668 provides data for most, but not all, of the "dioxin-like" PCBs, including those with the highest TEFs, as determined by the World Health Organization. This method provides detection limits frequently required in risk assessments.
Limitations	The GC/MS portions of this method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons. Method 1668 does not provide data for all of the "dioxin-like" PCBs, as does Method 1668A.
Reference	USEPA. 1997e Method 1668: Toxic Polychlorinated Biphenyls by Isotope Dilution HRGC/HRMS, EPA-821-R-97-001. Office of Water, U.S. Environmental Protection Agency, Washington, DC.
Website	http://www.epa.gov/clariton/clhtml/pubtitleOW.html — Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-13	
Method Title	Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, EPA Method 1668 Revision A	
Purpose	This method is for congener-specific determination of more than 150 chlorinated biphenyl (CB) congeners in solids (not tissue).	
Method Summary	This method is performance-based. A 20-g aliquot of sample is homogenized, and a 10-g aliquot is spiked with the labeled compounds. The sample is mixed with anhydrous sodium sulfate, allowed to dry for 12 - 24 hours, and extracted for 18- 24 hours using methylene chloride:hexane (1:1) in a Soxhlet extractor. The extract is evaporated to dryness, and the lipid content is determined. After extraction, a labeled cleanup standard is spiked into the extract which is then cleaned up using back-extraction with sulfuric acid and/or base, and gel permeation, silica gel, or Florisil chromatography. Activated carbon and high-performance liquid chromatography (HPLC) can be used for further isolation of specific congener groups. Prior to the cleanup procedures cited above, tissue extracts are cleaned up using an anthropogenic isolation column. After cleanup, the extract is concentrated to 20 µL. Immediately prior to injection, labeled injection internal standards are added to each extract and an aliquot of the extract is injected into the gas chromatography (GC). The analytes are separated by the GC and detected by a high-resolution ($\geq$10,000) mass spectrometer. Without interferences, EMDLs and EMLs will be, respectively, 0.5 and 1.0 ng/kg for soil, tissue, and mixed-phase samples, and EMLs for extracts will be 0.5 pg/uL. EMD: = Estimated Method Detection Limit; EML = Estimated Minimum Level	
Data Uses/Application	This method is for use in data gathering and monitoring associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.	
Advantages	Method 1668A provides congener data that can be used for source identification. Listed PCBs include the 12 World Health Organization “dioxin-like” PCBs. The HRMS method provides lower EMDLs compared to ECD or low resolution MS analyses and provides unequivocal congener identification.	
Limitations	The GC/MS portions of this method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons. Solvents, reagents, glassware, and other sample processing hardware may yield artifacts, elevated baselines, and/or lock- mass suppression causing misinterpretation of chromatograms. The natural lipid content of tissue can interfere in the analysis of tissue samples for the CBs.	
Reference	USEPA. 1999c. Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, EPA-821-R-00-002. Office of Water, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/region08/water/wastewater/biohome/biosolidsdown/methods/1668a5.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-14	
Method Title	Determination of Percent Dry Weight for Tissues	
Purpose	To determine the percentages of dry weight and moisture in tissue samples.	
Method Summary	A 0.5-1 g aliquot of homogenized sample is placed into a pre-weighed beaker and weighed. The samples are dried for 24 hours in a drying oven set at 63-65°C. Samples are placed in a desiccator and allowed to cool to room temperature for at least 30 minutes. The samples are weighed. The samples are put back in the oven for at least 2 hr after which they are removed from the oven and allowed to cool for at least 30 min in a desiccator. The sample is reweighed. If the difference between the first and second weighting is less than ± 0.02 g, the dry weight percent is calculated based on the last weighing. The difference between the weight of the dried sample and that of the wet sample is used to calculate the percent dry weight. $\text{Percent dry weight} = \frac{[\text{Vial wt. + Dry sample wt.}] - [\text{Vial wt.}]}{[\text{Vial wt. + Wet sample wt.}] - [\text{Vial wt.}]} \times 100$	
Data Uses/Application	Some exposure assessment models require concentration data on dry weight basis.	
Advantages	National database of fish and contaminant data reported on dry weight basis following this procedure.	
Limitations	None.	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	http://ccma.nos.noaa.gov/publications/tm130.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-15	
Method Title	Determination of Percent Lipid in Tissue	
Purpose	To determine the percent lipid (weight/weight basis) of a tissue.	
Method Summary	An appropriate amount of sodium sulfate-dried tissue sample is extracted three times with dichloromethane (100 mL each time). An aliquot of 20 mL of the extract is quantitatively removed for lipid determination. This aliquot is filtered, further dried with sodium sulfate, and brought to a final volume of 1 mL in dichloromethane. An aliquot of 100 µL was taken and evaporated to constant weight. The residual weight of this dried 100 µL portion is used to calculate the percent lipids of the sample based on the dry weight.	
Data Uses/Application	Lipid content has been found to be correlated to contaminant concentrations for specific tissues and whole organisms.	
Advantages	National database of fish and bivalve contaminant data reported on lipid basis following this procedure.	
Limitations	The Bligh and Dyer (1959) Method, using a different solvent system, provides slightly different lipid values.	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	http://ccma.nos.noaa.gov/publications/tm130.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.2-16
Method Title	Microwave Extraction of Marine Tissue for Semivolatile Organic Analytes, AED LOP 2.03.030, Revision 0
Purpose	Microwave-assisted extraction of semi-volatile organic compounds from marine tissue samples.
Method Summary	Homogenize the entire tissue sample using a tissue homogenizer. Determine dry weight to wet weight ratio. Weigh approximately 1.0 g of homogenized sample into preweighed aluminum pan. Place pan in drying oven and record weight at 24 and 48 hours. Using the dry/wet ratio, back-calculate the wet weight needed for each sample, setting the dry weight constant between 0.8 and 1.0 g target dry weight. Use the sample with the lowest dry/wet ratio (highest percent moisture) and back calculate the wet weight for that sample (see A). Since the moisture content is not the same for all the samples, the wet weight will also be different (see B). Adjust the wet weight of all samples to be equivalent to the standardization samples by adding hexane rinsed DI water (see C). A.: Target dry weight/(dry/wet ratio sample A) = grams wet sample A B: Target dry weight/(dry/wet ratio sample B) = grams wet sample B C: Grams wet sample A - Grams wet sample B = grams H_20 added to sample B. Assemble and prepare extraction vessels according to operation manual (AED uses a CEM MES-1000 microwave extraction system). Weigh samples directly into the bottom of the liners. Standardize the wet weight for all samples by adding hexane rinsed DI water. Add internal standards (IS) as required. For samples < 1 g, grind sample with 5 g of sodium sulfate and transfer to extraction vessel. Add 30 mL of 20/80 hexane/acetone solvent mixture, stir gently with a Teflon spatula, and insert the liner into a clean, dry, particle-free vessel body. Program the microwave at 70% power, 200 psi, 30 minutes runtime; 15 minutes at pressure, and 115° C. After the extraction, pour the top solvent layer from the extraction vessel into a pre-solvent rinsed 250 mL separatory funnel containing 80 mL of hexane rinsed DI water. Back extract the DI/acetone; hexane phase in the separatory funnel 3X with hexane, using 10mL hexane for the first extraction and 5 mL each for the second and third extractions. Combine the extracts and treat with sodium sulfate to remove water. Transfer the extract into a clean rinsed 200 mL Turbo-Vap® tube. Place the flask into the Turbo-Vap® apparatus and turn on the unit. Adjust the associated nitrogen pressure regulator to read approximately 5 psi. Reduce the sample volume to approximately 1 mL. Adjust the final volume to 1.0 mL with hexane. Remove 0.1 mL into a preweighed aluminum pan for lipid weight determination. Allow it to dry at room temperature for a minimum of 24 hours. Record the weight of the pan plus sample. Fractionate the sample using column chromatography with silicic acid.

Fact Sheet No.	2.3.2-16 (contd.)	
Data Uses/Application	Extracting semivolatile organic compounds from the tissue of aquatic fauna. Extracts can be further processed by separation on silicic acid chromatography procedures prior to analysis by gas chromatography and/or gas/chromatography/mass selective detector.	
Advantages	More time efficient and requires less solvent than other methods of semivolatile organic compound extraction from tissue, such as sonication or maceration in solvent.	
Limitations	This procedure was written to meet the specific needs of the research program at the U.S. EPS-Atlantic Ecology Division. It is not a U.S. EPA Standard Method and must not be referred to as such. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.	
Reference	Unpublished laboratory SOP, EPA NHEERL-AED, Narragansett RI	
Website	N/A	Last Accessed:

2.3.3 Biological Analysis Methods

Section 2.3.3 provides a compendium of biota-related biological analyses. Biota data like these are often used to measure the status and trends of environmental pollution on freshwater, estuarine, and marine macroinvertebrates to assess water quality criteria and monitor surface water quality. Thus, many of these methods pertain to the analysis of samples collected in the field to determine species abundance and taxa richness. Biological impairments resulting from pollution are often evaluated using indices derived from the sampling data that evaluate matrices such as community, population and functionality parameters. Other fact sheets pertaining to histopathological analyses are often included.

The sources of information for the biological analyses fact sheets come from the following agencies and offices:

- The USEPA's EMAP Program
- The USEPA's Environmental Research Laboratory-Narragansett,
- The USEPA's Great Lakes Program Office
- NOAA's Status and Trends Program
- The USEPA's Office of Water

Fact Sheet No.	2.3.3-1
Method Title	Laboratory Identification, Enumeration and Biomass Measurements of Periphyton in Wadeable Streams
Purpose	To determine periphyton species composition and/or biomass in the laboratory
Method Summary	The standard laboratory-based method provides the option of sampling natural substrates in a few different ways. Regardless of the sampling strategy, the samples are returned to the laboratory where they are homogenized, sorted, identified and counted in order to derive relative abundance and taxa richness data. "Soft" (non-diatom) samples are homogenized with a tissue homogenizer or a blender. These thoroughly mixed samples are placed in Palmer counting cells. Approximately 300 algal "cell units" are counted and identified to the lowest possible taxonomic level at 400x magnification. Relative abundances of soft algae are determined by dividing the number of cells counted for each taxon by the total number of cells counted. Diatom samples are subsampled and oxidized. The diatoms are then mounted on a high refractive index medium to make permanent slides. Diatom valves are counted and identified to the lowest possible taxonomic level, which should be species and perhaps variety level, under oil immersion at 1000X magnification. At minimum, count 600 valves (300 cells) and at least until 10 valves of 10 species have been observed. Relative abundances of diatoms have to be corrected for the number of live diatoms observed in the count of all algae. To determine the relative abundance of diatom species in the algae assemblage, divide the number of valves counted for each species by the total number of valves counted; then multiply the relative abundance of each diatom taxon in the diatom count by the relative abundance of live diatoms in the count of all algae. USEPA's EMAP document describes a similar method for enumerating and measuring periphyton from wadeable streams (USEPA, 1998).
Data Uses/Application	Species relative abundance and taxa richness are data derived from these protocols. These data parameters provide information pertaining to the status and trends of environmental pollution and its impacts on freshwater, marine and estuarine communities. Biological impairment resulting from pollution is often evaluated using metrics of biotic integrity derived from the aforementioned data parameters that evaluate community, population and functional parameters. Examples of metrics based on species composition include species richness, total number of genera, total number of divisions, shannon diversity (for diatoms), percent community similarity of diatoms, pollution tolerance index for diatoms, and percent sensitive diatoms. Furthermore, other metrices infer ecological conditions based on documented preferences. These metrices include the percent aberrant diatoms, percent motile diatoms, simple diagnostic metrics, inferred ecological conditions with simple autecological indices (SAI), inferred ecological conditions with weighted average indices, and impairment of ecological conditions.

Fact Sheet No.	2.3.3-1 (contd.)	
Advantages	The laboratory-based survey is more accurate in assessing biotic integrity and in diagnosing causes of impairment than the field-based survey.	
Limitations	The laboratory-based methods require more time and effort than the field evaluation.	
Reference	Barbour *et al.* 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition, EPA 841-B-99-002. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/owowwtr1/monitoring/rbp/index.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.3-2	
Method Title	Laboratory Periphyton Biomass Determination	
Purpose	To determine periphyton biomass in the laboratory.	
Method Summary	To quantify algal biomass, the area of the substrate sampled must be determined. Periphyton biomass can be estimated with chlorophyll *a*, ash-free dry mass, cell densities, and biovolume, usually per cm^2. Each of these measures estimates a different component of periphyton biomass. Chlorophyll *a*: Extract in acetone and measure chl concentration in the extract with a spectrophotometer or fluorometer. Calculate the chlorophyll *a* density on substrates by determining the proportion of original sample that was assessed for chlorophyll *a*. Ash-Free Dry Mass: A measurement of the organic matter in samples. At detailed description of the process is beyond the scope of this fact sheet, but standard methods are readily available (APHA, 1999, USEPA 1995). It is a fairly simple analysis. It is recommended over dry mass measurements because silt can account for a substantial proportion of dry mass in some samples. Area-Specific Cell Densities and Biovolumes: Cell densities are determined by dividing the numbers of cells counted by the proportion of sample counted and the area from which the samples were collected. Cell biovolumes are determined by summing the products of cell density and biovolume of each species counted and dividing that sum by the proportion of sample counted and the area from which the samples were collected. USEPA EMAP provides similar guidance for laboratory periphyton biomass determination (USEPA, 1998).	
Data Uses/Application	Biomass may be especially important in studies that address nutrient enrichment or toxicity.	
Advantages	Periphyton biomass provides information on standing crop, which is useful for assessing the biological integrity of streams.	
Limitations	In many cases, sampling benthic algae misses peak biomass, which may best indicate nutrient problems and potential for nuisance algae growths.	
Reference	Barbour *et al.* 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. USEPA. Office of Water. Washington DC.	
Website	http://www.epa.gov/owowwtr1/monitoring/rbp/index.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.3-3
Method Title	Laboratory Analysis of Benthic Macroinvertebrates in Wadeable Streams
Purpose	To determine macroinvertebrate diversity and abundance.
Method Summary	Following sample collection, the sediment is sieved and preserved in a 10% buffered formalin solution, however different mixtures should be used for soft-bodied organisms (*i.e.,* leeches, aquatic oligochaete, and other soft-bodied organisms. Samples are sorted by hand in the laboratory using a low power (2x) scanning lens or a stereomicroscope. Approximately one or two tablespoonfuls of the sample are placed in a white enamel pan filled about one-third full of water. Ethanol-preserved organisms should float to the top and be removed from the dish as a sub-sampling procedure. Various staining methods may also be used for sub-sampling. Microscope slide mounts are then prepared for all or parts of organisms for identification purposes. These slides are then identified to a specific taxonomic level. For water quality and pollution analyses, it is important that organisms are identified to the species level. As organisms are identified, the individuals in each taxonomic category are counted and the numbers are recorded in laboratory bench sheets. Subsampling of benthic samples is not a requirement and is often discouraged by certain scientists. However, Rapid Bioassessment Protocols recommend a fixed-count approach to subsampling and sorting the organisms based on the sample matrix of detritus, sand and mud. This approach calls for sieving samples as described above and then removing all material in four randomly selected grids contained the sieved sample. The organisms in these four grids are enumerated and identified to the lowest possible taxonomic level. Several USEPA EMAP documents describe similar protocols to determine macroinvertebrate diversity and abundance (USEPA, 1990b; USEPA, 2000b; USEPA, 1995).
Data Uses/Application	Macroinvertebrate data such as these are used to measure the status and trends of environmental pollution. Biological impairment resulting from pollution is often evaluated using indices derived from the sampling data that evaluate matrices such as community, population and functional parameters.
Advantages	Examples of indices often derived from the data include the Hilsenhoff's Family Biotic Index (HBI), Invertebrate Community Index (ICI), Community Similarity Indices, Community Loss Index, and the Ohio EPA Invertebrate Community Index.
Limitations	Laboratory analysis of benthic macroinvertebrate samples is costly and requires a high degree of taxonomic expertise.

Fact Sheet No.	2.3.3-3 (contd.)	
Reference	Barbour *et al.* 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition, EPA 841-B-99-002. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/owowwtr1/monitoring/rbp/index.html	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.3-4
Method Title	Laboratory Analysis of Water Column Organisms
Purpose	To determine composition and abundance from drift net or stream-net samplers.
Method Summary	The organism's collected by drift nets or stream-net samplers are emptied directly into a white enamel pan or small bucket. The organisms can then be hand-picked into a sample container and filled three-fourths full of preservative (70-80% ethyl alcohol), however different mixtures should be used for soft-bodied organisms (*i.e.,* leeches, aquatic oligochaete, and other soft-bodied organisms). Sample containers should be large enough so that they are not over one-half full of the washed sample before the preservative is added. Samples should be sorted by hand in the laboratory using a low power (2x) scanning lens or a stereomicroscope). Approximately one or two tablespoonfuls of the sample will be placed in a white enamel pan filled about one-third full of water. Ethanol-preserved organisms should float to the top and be removed from the dish as a sub-sampling procedure. Various staining methods have also been used for sub-sampling. Microscope slide mounts are then prepared for all or parts of organisms for identification purposes. These slides are then identified to a specific taxonomic level depending on the needs, experience and available resources. For water quality and pollution analyses, it is important that organisms are identified to the species level. As organisms are identified, the individuals in each taxonomic category are counted and the numbers are recorded in laboratory bench sheets.
Data Uses/Application	Macroinvertebrate data such as these are used to measure the status and trends of environmental pollution on freshwater, estuarine, and marine macroinvertebrates, to assess water quality criteria, and monitor surface water quality. Biological impairment resulting from pollution is often evaluated using indices derived from the sampling data that evaluate matrices such as community, population and functional parameters. Examples of indices often applied include the Hilsenhoff Biotic Index (HBI), Invertebrate Community Index (ICI), Community Similarity Indices, Community Loss Index, and the Ohio EPA Invertebrate Community Index .
Advantages	Macroinvertebrate biomass (weight of organisms per unit area) is a useful measure of standing crop which is useful in assessing the biological integrity of surface waters. The results of this analysis feed into the commonly accepted incdices used to evaluate community health.
Limitations	Taxonomic analysis requires a team of highly experienced technicians and/or scientists.
Reference	USEPA. 1990b. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA/600/4-90/030. Office of Research and Development, Washington, D.C.

Fact Sheet No.	2.3.3-4 (contd.)	
Website	http://www.epa.gov/clariton/clhtml/pubtitleORD.html	Last Accessed: 2/13/03

Fact Sheet No.	2.3.3-5	
Method Title	SOP-2: Lab Analysis of Lake Trout Stomachs and Data Entry; Appendix B. Standard Operating Procedure for Lab Analysis of Coho Salmon Stomachs and Data Entry, LMMB 026 - Appendix 2 & LMMB 027 - Appendix B	
Purpose	To examine and quantify the contents of lake trout and coho salmon stomachs.	
Method Summary	Lake trout stomachs: Prey fish in the stomachs are identified, measured (nearest mm) and weighed (nearest 0.1 kg). The percent digested state is recorded. Measures of length include: maximum total length, standard length, vertebral column length, and length of a multiple of 5 vertebrae. Fish or parts of fish that cannot be positively identified are recorded as unidentified remains. Invertebrates are identified, grouped by taxa, and weighed as a taxon group. The number of individuals in each group is enumerated. Stomach contents are repackaged and frozen. Using the weight and length of intact prey, conversion equations are developed to reconstruct total prey length and weight from partial length measures. Coho salmon stomachs: The stomach is rinsed with rinse water to remove excess formalin. Prey fish in the stomachs are identified, measured (nearest mm) and weighed (nearest 0.1 g (large items and 0.02 g for small items). The percent digested state is recorded. Measures of length include: maximum total length, standard length, vertebral column length, and length of as many vertebrae as possible. Invertebrates are identified, grouped by taxa, and weighed as a taxon group. The number of individuals in each group is enumerated. The average length and digested state of each taxon group is recorded. If identification of a prey item is uncertain, the item is examined by a second identifier and compared to a reference collection of diet items. During the analysis, examples of each species of prey fish and taxonomic group of invertebrate is set aside and preserved in 5% formalin. Stomach contents are repackaged and preserved. Using the weight and length of intact prey, conversion equations are developed to reconstruct total prey length and weight from partial length measures. Standard Method 10600D.2 also discusses diet analysis of fish (APHA, 1999).	
Data Uses/Application	Stomach content data of upper trophic level fish are important to understand exposure pathways.	
Advantages	Standardized method enhances uniformity of data.	
Limitations	N/A	
Reference	USEPA. 1997b. Methods LMMB 026 - Appendix 2 & LMMB 027 - Appendix B. Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL	
Website	http://www.epa.gov/glnpo/lmmb/methods/qappfish.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.3-6	
Method Title	Gonadal Analysis	
Purpose	A method to determine the reproductive stage of oysters, mussels, and zebra mussels.	
Method Summary	A semi-quantitative histological approach is used to rank reproductive stage. For oysters and mytilid mussels, a dorsal-ventral slice of tissue is taken and fixed in Davidson's fixative (48 hours for oysters and 1 week for mussels). Zebra mussels are fixed whole in Davidson's fixative for one week, decalcified with acetic acid, and embedded whole. Tissue samples are embedded in paraffin, sectioned at a 5-µm thickness, and stained using a pentachrome staining protocol. Unstained sections may be used for histopathological analysis. Stained sections are examined under a compound microscope. Sex and state of gonadal development are determined. The stage of the gametogenic cycle is assigned a numerical value. For mytilids and zebra mussels, a mean gonadal index, ranging from 0 to 5, can be calculated by summing the individual stage numbers. For oysters, the number of individuals with substantial gonadal development are compared to those having little gonadal volume using an egg/eggless ratio.	
Data Uses/Application	This method helps to assess the physiological state of bivalve populations. Analysis of reproductive stage is important in identifying differences in tissue composition which might affect between site and interannual comparisons of contaminant data.	
Advantages	The pentachrome staining procedure yields better differentiation of tissue types and mucins. This analysis provides an assessment of sexual stage in the gametogenic cycle, and if desired, allows for a concomitant histopathological analysis, with a single sample preparation protocol.	
Limitations	The procedure cannot be performed on pooled samples, as is commonly done for chemical parameters, but only on individuals.	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	http://ccma.nos.noaa.gov/publications/tm130.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.3-7	
Method Title	Histopathological Evaluations of Target and Non Target Fish Species	
Purpose	To determine fish quality in Estuaries	
Method Summary	Specimens for histopathology analysis are unpacked, logged in, and placed in 70% ethyl alcohol for at least 48 hours prior to examination. A careful visual inspection is made of the fins and body surfaces. Any abnormalities are noted. Thorough examinations of the eyes, branchial chambers, buccal cavity, visceral organs is performed. Representative tissue samples are removed from either fish or shellfish (USEPA and the Naval Construction Battalion Center, 1992) and slides are prepared. These slides are then examined using a compound research microscope to diagnose pathological conditions.	
Data Uses/Application	Histopathological evaluations provide data that can be used as a composite index of the incidence of diseases and contaminant body burdens in selected resident species. Microscopic examination can determine the presence or absence of pathological changes and evaluate the health of the animal or its exposure to contaminated material or infectious agents. Changes include morphological alterations, variations in the normal staining characteristics, or a change in the rate of occurrence of features (*i.e.,* mitotic figures)	
Advantages	Histopathological investigations provide information on the relationship between incidence of external abnormalities and internal histopathological abnormalities.	
Limitations	It is difficult to prove in a legal context that both external and internal abnormalities are indicators of degraded environmental systems.	
Reference	USEPA. 1995. Environmental Monitoring and Assessment Program (EMAP), Laboratory Methods Manual, Estuaries, Volume 1-Biological and Chemical Analyses, EPA/620/R-95/008. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.	
Website	http://www.epa.gov/emap/html/pubs/docs/groupdocs/estuary/field/lab_man.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.3-8	
Method Title	Histopathology Analysis	
Purpose	A quantitative or semi-quantitative method to determine the prevalence and density of parasites, pathologies, and diseases afflicting oysters, mussels, and zebra mussels.	
Method Summary	Analyses are conducted on paraffin-embedded tissues sectioned at a 5-µm thickness and stained using a pentachrome staining procedure. Prepared slides are examined individually under the microscope using a 10X ocular and a 10X objective. Conditions evaluated are scored for intensity using either a quantitative or semi-quantitative scale. Conditions scored quantitatively include parasites, the number of ceroid bodies, incidences of tissue inflammation, rickettsial bodies, incidences of tissue edema, and suspected neoplasms and tumors. A running count of incidences of the condition is kept as the slide is scanned, to avoid re-examining each slide multiple times for each separate malady. Evaluation of conditions scored semi-quantitatively related to the intensity of the effect or the extensiveness of pathologies affecting large tissue areas. Semi-quantitative measurement may require re-scanning portions of the tissue for each malady type to completely assess the degree of tissue involvement. Infection intensity of parasites, the occurrence and extensiveness of tissue pathologies, and the intensity of diseases are recorded using semi-quantitative or quantitative measures.	
Data Uses/Application	Histopathology is used to help assess the influence of contaminant exposure on population health.	
Advantages	Infection intensity quantified by counts or semi-quantitative methods consistently provides a more robust data set for statistical analysis.	
Limitations	Prevalence rarely provides an adequate description of the population dynamics of disease and often yields ambiguous results.	
Reference	NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.	
Website	http://ccma.nos.noaa.gov/puslications/tm130.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.3-9	
Method Title	Index of Biotic Integrity (IBI)	
Purpose	The IBI is used to identify and assess degraded and undegraded streams.	
Method Summary	The steps in developing IBIs are the same for both fish and benthic macroinvertebrates. Criteria for both reference and degraded sites were determined based on water chemistry, physical habitat, and land use. Ecologically-relevant geographic strata were determined using cluster analysis and nonmetric multidimensional scaling. Candidate metrics were evaluated for 1) their ability to discriminate (based on classification efficiency) between reference and degraded sites, and 2) for redundancy. The final suite of metrics used in the IBIs contained those ecologically significant metrics with the best classification efficiency. Both IBIs were validated using an independent data set and overall classification efficiencies were calculated. An example of the metrices developed for the Fish Index of Biotic Integrity are provided below: • Number of native species • Number of benthic species • % tolerant individuals • % abundance of dominant species • % generalists, omnivores and insectivores • Number of individuals/square meter • Biomass (grams/square meter) (used for coastal plain streams only) • % lithophilic spawners • % insectivores (used for non-coastal plain streams only)	
Data Uses/Application	IBIs are used to determine biological integrity based on characteristics of the fish and benthic assemblage at a site. The results of these assessments are used for watershed management decisions concerning strategies that will control and minimize point and non-point sources of water pollution.	
Advantages	IBIs use multiple attributes to quantitatively assess stream health. It is a systematic way in which to interpret data for management decisions.	
Limitations	Different states have developed similar, yet different IBIs. This makes it difficult to compare the results from one state to another.	
Reference	USEPA. 1997f. State of the Streams: 1995-1997 Maryland Biological Stream Survey Results, Mid-Atlantic Integrated Assessment. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.dnr.state.md.us/streams/pubs/ea-99-6.pdf	Last Accessed: 1/31/2003

Fact Sheet No.	2.3.3-10	
Method Title	Fish Bioassessment I and II	
Purpose	These bioassessment strategies use existing and new information in a systematic manner to determine the health of the local fish community.	
Method Summary	Fish Bioassessment I uses a questionnaire to serve as a screening tool to pool the existing knowledge regarding fishery populations and health from the local fish community. The questionnaire polls state fish biologists and university ichthyologists believed to be knowledgeable about the fish assemblages in stream reaches of concern. Potential respondents are contacted initially by telephone to identify appropriate respondents. Then the questionnaire is mailed to all respondents for completion followed by follow-up mailings and telephone contact. Questionnaire responses should provide information pertaining to the integrity of the fish community, the frequency of limiting factors and causes, the frequency and occurrence of particular fish community condition characterizations, the geographic patterns of these variables, the temporal trends in the variables, the effects of water body type and size on the spatial and temporal trends, the likelihood of improvement and degradation and the major limiting factors. The data are then analyzed and results are reported as histograms, pie graphs, or box plots. Based on the results of the Bioassessment I survey, Bioassessment II pursues standardized field collection, species identification and enumeration, and community analyses using biological indices or quantification of the biomass and numbers of key species.	
Data Uses/Application	The questionnaire provides a qualitative assessment of a large number of water bodies quickly an inexpensively. Its quality depends on the survey design, the questions presented, and the knowledge and cooperation of the respondents. The fish Bioassessment II survey yields an objective, discrete measure of the health of the fish community. Data provided in this survey can be used to develop biological criteria, prioritize sites for further evaluation, provide a reproducible impact assessment, and monitor trends in fish community status.	
Advantages	Questionnaires can provide information that field surveys cannot such as historical trends and conditions. Field surveys can be oriented towards gathering data in areas where data were missing historically.	
Limitations	Questionnaires are sometimes inaccurate due to hasty responses, they often report conditions better than they are in reality, and the respondents have insufficient knowledge to answer the questions.	
Reference	USEPA. 1993c. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/R-92. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.	
Website	http://www.epa.gov/bioindicators/html/fish_methods.html	Last Accessed: 1/31/2003

3.0 REFERENCES

APHA. 1999. Standard Methods for the Examination of Water and Wastewater, 20th Edition, L.S. Clesceri and A.D. Eaton, Eds., American Public Health Association, Washington D.C.

ASTM. 2001a. ASTM Book of Standards. Volume 11.01. American Society for Testing and Materials, West Conshocken, PA.

ASTM. 2001b. ASTM Book of Standards. Volume 11.05. American Society for Testing and Materials, West Conshocken, PA.

ASTM. 2001c. ASTM Book of Standards. Volume 11.02. American Society for Testing and Materials, West Conshocken, PA.

ASTM. 2001d. ASTM Book of Standards. Volume 03.06. American Society for Testing and Materials, West Conshocken, PA.

ASTM. 2001e. ASTM Book of Standards. Volume 04.08. American Society for Testing and Materials, West Conshocken, PA.

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. Office of Water, U.S. Environmental Protection Agency; Washington, D.C.

Battelle 2001 Natural Recovery of Persistent Organics in Contaminated Sediments at the Sangamo-Weston/Twelvemile Creek/Lake Hartwell Superfund Site. USEPA National Risk Management Research Laboratory Cincinnati, OH.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology.* 37:911.

Brannon, J.M., R.M. Engler, J.R. Rose, P.G. Hunt, and I. Smith. 1976. Selective Analytical Partitioning of Sediments to Evaluate Potential Mobility of Chemical Constituents During Dredging and Disposal Operations, WES Technical Report D-76-7. U.S. Army engineer Waterways Experiment Station, Vicksburg MS.

Carr, R.S. 2001. Marine and Estuarine Sediment Quality Assessment Studies. Proceedings of the U.S. Geological Survey (USGS) Sediment Workshop, February 4-7, 1997. Available at http://water.usgs.gov/osw/techniques/workshop/carr.html. September 27, 2001.

Centre for Environment, Fisheries and Aquaculture Science (CEFAS) 2002. Guidelines for the conduct of benthic studies at aggregate dredging sites. Department for Transport, Local Government and the Regions, London, U.K.

Custer, C.M., T.W. Custer, P.D. Allen, K.L. Stromborg, and M.J. Melancon. 1998. Reproduction and environmental contamination in tree swallows nesting in the Fox River drainage in Green Bay, Wisconsin, USA. *Environmental Toxicology and Chemistry.* 17:1786-1798.

Elonen, Gregory E., RL Spehar, GW Holcombe, RD Johnson, JD Fernandez, RJ Erickson, JE Tietge, and PM Cook. 1998. Comparative Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin to Seven Freshwater Fish Species During Early Life-Stage Development. Environmental Toxicology and Chemistry, Vol. 17, No. 3, pp 472-483

Environment Canada. 1990. Acute Lethality Test Using Rainbow Trout. EPS 1/RM/9 Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 1990. Acute Lethality Test Using *Daphnia spp.*. EPS 1/RM/11 Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 1992. Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*. EPS 1/RM/21. Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 1992. Test of Larval Growth and Survival Using Fathead Minnows. EPS 1/RM/22 Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 1992. Growth Inhibition test Using the Freshwater Alga *Selenastrum capricornutum*. EPS 1/RM/25. Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 1992. Fertilization Assay Using Echinoids. EPS 1/RM/27. Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 1997. Test for Survival and Growth in Sediment Using the Larvae of freshwater Midges (*Chironomus tentans* or *Chironomus riparius*). EPS 1/RM/32. Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 1997. Test for Survival and Growth in Sediment Using the Freshwater Amphipod *Hyalella azteca* . EPS 1/RM/33 Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 1998. Toxicity Tests Using Early Life Stages of Salmonid Fish. EPS 1/RM/28, 2nd edition. Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 1999. Test for Measuring the inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*. EPS 1/RM/37. Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 2001. Test for Survival and Growth in Sediment Using the Spionid Polychaete Worms (*Polydora cornuta*). EPS 1/RM/41. Environmental Protection Service, Environment Canada, Ottawa, Ontario, Canada.

Fitzgerald, S.A., J Val Klump, PW Swarzenski, RA Mackenzie, and KD Richards. 2001. Beryllium-7 as a Tracer of Short-Term Sediment Deposition and Resuspension in the Fox River, Wisconsin. *Environmental Science and Technology* 35/ 300-305.

Folk, R.L. 1974. Petrology of Sedimentary Rocks. Hemphill Publishing Company, Austin TX. 182 pp.

Ho, K.T, A. Kohn, M. Pelletier, H. Mcgee, R. M. Burgess, and J. Serlost. 2000. Sediment Toxicity Assessment: Comparison of standard and new test designs. *Archives of Environmental Contamination and Toxicology.* 39, 462-468.

Ho, K.T., R. A. Kinney, A. Kuhn, M.C. Pelletier and R. M Burgess. 1997. Identification of Acute Toxicants in New Bedford Harbor Sediments. *Environmental Toxicology and Chemistry*. 16, 551-558.

Hunt, C.D., D. Redford, H. White, A. Robertson, F. Aikman III, and D. Pabst. Transport, Fate, and Effects of Sewage Sludge Disposal at the 106-Mile Site: A Summary and Synthesis of Findings. *Journal of Marine Environmental Engineering*, 3. 313 - 326.

Ireland, D.S., Burton, G.A. and G.G. Hess. 1996. *In Situ* Toxicity Evaluations of Turbidity and Photoinduction of Polycylic Aromatic Hydrocarbons. *Environmental Toxicology and Chemistry*. Vol. 15: 4. p 574-581.

Lipscomb, S.W. 1995. Quality Assurance Plan for Discharge Measurements Using Broadband Acoustic Doppler Current Profilers. USGS Open-File Report 95-701.

Long E.R., D.D. Mac Donald, S.L. Smith, and F.D. Calder. 1995. Incidence of Adverse Biological Effects with Ranges of Chemical Concentrations in Marine and Estuarine Waters. *Environmental Management* 19 (1): 81-97.

Long, E.R. and L.G. Morgan. 1990. The Potential for Biological Effects of Sediment-Sorbed Contaminants Tested in the National Status and Trends Program. NOAA Tech. Memo. NOS OMA 62. National Oceanic and Atmospheric Administration, Seattle WA.

MacDonald D.D., C.G. Ingersoll, and T.A. Berger. 2000. Development and Evaluation of Consensus-Based Sediment Quality Guidelines for Freshwater Ecosystems. *Archives of Environmental Contamination and Toxicology* 39: 20-31.

Manheim F.T. 1966. A Hydraulic Squeezer for Obtaining Interstitial Water from Consolidated and Unconsolidated Sediments. Geol. Surv. Prof. Pap. U.S., 550-C: 256 - 261.

Murdoch, Alena and Jose M. Azcue. 1995. Manual of Aquatic Sediment Sampling. Lewis Publishers, Boca Raton, FL. 219 pp.

NFESC. 2000. Rapid Characterization of Metals in Sediments Using X-Ray Fluorescence (XRF) Technology. Naval Facilities Engineering Command, Washington DC.

NOAA. 1998. Sampling and Analytical Methods of the National Status and Trends Program, Mussel Watch Project: 1996 Update, NOAA Technical Memo NOS ORCA 130. National Oceanic and Atmospheric Administration, Silver Spring, MD. 233 pp.

PSWQAT. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound, Puget Sound Protocols and Guidelines. Puget Sound Water Quality Action Team, Olympia, WA.

Sarda, N. and G.A. Burton. 1995. Ammonia Variation in Sediments: Spatial, Temporal, and Method-Related Effects. *Environmental Toxicology and Chemistry*. Vol 14: 9.

Sasson-Brickson, G. and G.A. Burton, Jr. 1991. *In Situ* and Laboratory Sediment Toxicity Testing with Ceriodaphnia Dubia. *Environmental Toxicology and Chemistry.* Vol 10. P 201-207.

Sediment Management Work Group. 1999. Contaminated Sediment Management Technical Papers. Sediment Management Work Group, Detroit MI.

USACE/WDNR/WDEC. 2000. Dredged Material Evaluation and Disposal Procedures, A Users Manual for the Puget Sound Dredged Disposal Analysis (PSDAA) Program U.S. Army Corps of Engineers, Seattle District, U.S. Environmental Protection Agency Region 10, Washington State Department of Natural Resources, Washington State Department of Ecology

USACE Engineering and Design.1996. Soil Sampling Engineering Manual. EM 1110-1-1906. Department of the Army, Washington D.C.

US Army Engineer Waterways Experiment Station. 1994. The Plume Measurement System (PLUMES), A Commercially Available System, Dredging Research Technical Notes DRP-1-16. Waterways Experiment Station, U.S. Army Corps of Engineers, Vicksburg, MS.

US Army Engineer Waterways Experiment Station. 1991. Hydrologic Surveys Applicable to Dredging Operations, Dredging Research Technical Notes DRP-2-03. Waterways Experiment Station, U.S. Army Corps of Engineers, Vicksburg, MS.

US Army Engineer Waterways Experiment Station. 1989. Monitoring Dredged Material Consolidation and

Settlement at Aquatic Disposal Sites. Environmental Effects of Dredging Technical Notes EEDP-01-5. Waterways Experiment Station, U.S. Army Corps of Engineers, Vicksburg, MS.

US Army Engineer Waterways Experiment Station. 1988. Acoustic Tools and Techniques for Physical Monitoring of Aquatic Dredged Material Disposal Sites. EEDP-01-10. Waterways Experiment Station, U.S. Army Corps of Engineers, Vicksburg, MS.

USEPA. 2001a. Method 245.7: Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Draft, EPA 821-R-01-008. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2001b. Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS, EPA 821-R-01-020. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2001c. Method 1632, Revision A: Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry, EPA-821-R-01-006. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2001d. Appendix to Method 1631: Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation, EPA-821-R-01-013. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2001e. Method for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-associated Contaminants with the Amphipod *Leptocheirus plumulosus.* EPA 600/R-01/020. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2000a. Bioaccumulation Testing and Interpretation for the Purpose of Sediment Quality Assessment: Status and Needs, EPA 823-R-00-001. Office of Water and Office of Solid Waste, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2000b. Coastal 2000 Northeast Component: Field Operations Manual, Environmental Monitoring and Assessment Program (EMAP), EPA/620/R-00/002. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 2000c. Method 6200, Field Portable X-Ray Fluorescence Spectrometry for the Determination of Elemental Concentrations in Soil and Sediment. Office of Solid Waste, U.S. Environmental Protection Agency, Washington DC.

USEPA. 2000d. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, EPA/600/R-99/064. Office of Science and Technology, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1999a. National Recommended Water Quality Criteria-Correction, EPA-822-Z-99-001. Office of Water, U.S. Environmental Protection Agency, Washington D.C.

USEPA. 1999b. Method 1631, Revision B: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, EPA 821-R-99-005. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1999c. Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, EPA-821-R-00-002. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1999d. Innovative Technology Verification Report: Aquatic Research Instruments Russian Peat Borer, EPA/600/R-01/010. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1999e. Sediment Sampling Technology: Art's Manufacturing & Supply, Inc. Split Core Sampler for Submerged Sediments, Superfund Innovative Technology, Evaluation Program. EPA/600/R-01/009. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1998. EMAP: Surface Waters, Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams. EPA/6201/R-94/004F. Office of Research and Development. U.S. Environmental Protection Agency, Washington, DC.

USEPA and USACE. 1998. Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S.-Testing Manual, Inland Testing Manual, EPA 823-B-98-004. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1997a. Ecological Risk Assessment Guidance for Superfund: Process for designing and Conducting Ecological Risk Assessments, Interim Final. EPA 540-R-97-006. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington D.C.

USEPA. 1997b. Lake Michigan Mass Balance Study Methods Compendium, Volume 1: Sample Collection Techniques, EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.

USEPA. 1997c.Lake Michigan Mass Balance Study Methods Compendium, Volume 3: Metals, Conventionals, Radio chemistry, and Biomonitoring Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.

USEPA. 1997d. Lake Michigan Mass Balance Study Methods Compendium, Volume 2: Organic and Mercury Sample Analysis Techniques. EPA 905-R-97-012c. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.

USEPA. 1997e. Method 1668: Toxic Polychlorinated Biphenyls by Isotope Dilution HRGC/HRMS, EPA-821-R-97-001. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1997f. State of the Streams: 1995-1997 Maryland Biological Stream Survey Results, Mid-Atlantic Integrated Assessment. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.

USEPA. 1996a. Ecological Significance and Selection of Candidate Assessment Endpoints. EPA 540-F-95-037. ECO Update (January 1996). Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington D.C.

USEPA. 1996b. Method 1639: Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption, EPA 821-R-96-006. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1996c. Method 1637: Determination of Trace Elements in Ambient Waters by Off-Line Chelation Pre-concentration and Stabilized Temperature Graphite Furnace Atomic Absorption, EPA 821-R-96-004. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1996d. Method 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma-Mass Spectrometry, EPA 821-R-96-005. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1996e. Method 1640: Determination of Trace Elements in Ambient Waters by On-Line Chelation Pre-concentration and Inductively Coupled Plasma-Mass Spectrometry, EPA 821-R-96-007. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1996f. Method 1632: Inorganic Arsenic in Water by Hydride Generation Quartz Furnace Atomic Absorption. EPA 821-R-96-013. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1996g. Method 1636: Determination of Hexavalent Chromium by Ion Chromatography. EPA 821-R-96-003. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1996h. Screening of Polychlorinated Biphenyls by Immunoassay, SW846 Method 4020. Office of Solid Waste, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1995. Environmental Monitoring and Assessment Program Laboratory Methods Manual, Estuaries, Volume 1-Biological and Chemical Analyses, EPA/620/R-95/008. Environmental Monitoring and Assessment Program , U.S. Environmental Protection Agency, Washington DC.

USEPA. 1994a. Field Studies for Ecological Risk Assessment. EPA 540-F-94-014. ECO Update (September 1994). Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington D.C.

USEPA. 1994b. Compendium of Environmental Response Team Standard Operating Procedures. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Edison, NJ.

USEPA. 1994c. Method 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, EPA 821-B-94-005. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1994d. Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods. EPA/600/R-94/025. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1993a. Biological and Chemical Assessment of Contaminated Great Lakes Sediment, EPA 905-R93-006. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.

USEPA. 1993b. Assessment and Remediation of Contaminated Sediments (ARCS) Program: Biological and Chemical Assessment of Contaminated Great Lakes Sediment, EPA 905-R93-006. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.

USEPA. 1993c. Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters, EPA/600/R-92-111. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1992a. Sediment Classification Methods Compendium. EPA/823-R-92-006. Office of Water, U.S. Environmental Protection Agency, Washington DC.

USEPA. 1992b. Framework for Ecological Risk Assessment. EPA/630/R-92/001. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington D.C.

USEPA. 1992c. Monitoring Guidance for the National Estuary Program. EPA 842-B-92-004. Office of Water. Office of Wetlands, Oceans and Watersheds. Washington. D.C.

USEPA. 1991. Draft Analytical Method for Determination of Acid Volatile Sulfide in Sediment, EPA-821-R-91-100. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1990a. Environmental Monitoring and Assessment Program: Near Coastal Component, 1990

Demonstration Project, Field Operations Manual. DRAFT. Contract #. 68-C8-0066. Office of Research and Development. Narragansett, Rhode Island.

USEPA. 1990b. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.

USEPA. 1989a. Method 1624, Revision B: Volatile Organic Compounds by Isotope Dilution GC/MS, EPA440-1-89-100. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA. 1989b. Method 1625, Revision B: Semi-volatile Organic Compounds by Isotope Dilution GC/MS, EPA440-1-89-100. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA and the Naval Construction Battalion Center. 1992. Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies. Technical Document 2296, Naval Command, Control and Ocean Surveillance Center, RDT&E Division, San Diego, CA.

USEPA and USACE. 1992. Guidance for Performing Tests on Dredged Material Proposed for Ocean Disposal. (Draft). U.S. Army Corps of Engineers, New York District and U.S. Environmental Protection Agency, Region II, New York, New York.

USEPA and USACE. 1991. Evaluation of Dredged Material Proposed for Ocean Disposal, Testing Manual, EPA 503-8-91-001. Office of Water, U.S. Environmental Protection Agency, Washington D.C.

USGS. 2001. The Krupaseep. Next Generation Seepage Meter. http://sofia.usgs.gov/sfrsf/entdisplays/krupaseep/. Updated July 23, 2001.

USGS. 1998. Tree Swallow Sample Collection and Processing Procedures, Technical Operating Procedure WE-410.0. Upper Mississippi Science Center, U.S. Geological Survey, LaCrosse, WI.

Weber, C.I. 1991. Methods for Measuring Acute Toxicity of Effluents in Receiving Waters for Freshwater and Marine Organisms. 4th edition. EPA/600/4-901027. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Index

Fact Sheet # 2.1.3 – 4	Method No. ERT SOP 2025: Chronic Freshwater Crustacean Bioassay (7day)
Fact Sheet # 2.1.3 - 5	Method No. ERT SOP 2028: Chronic Freshwater Crustaceans Bioassay (10 days)
Fact Sheet # 2.1.3 - 8	Method No. NHEERL-AED SOP 1.03.003: Acute Marine Crustacean Bioassay
Fact Sheet # 2.1.3 - 10	Method No. NHEERL-AED SOP 1.03.005: Chronic Estuarine Survival, Growth, and Fecundity Test
Fact Sheet # 2.1.3 - 13	Toxicity Evaluations of Photoinduction of Polycyclic Aromatic Hydrocarbons (PAH): In Situ Analysis
Fact Sheet # 2.1.3 - 14	Toxicity Evaluations of Photoinduction of Polycyclic Aromatic Hydrocarbons: Laboratory Analysis of Storm water

bioassay – echinoderm

Fact Sheet # 2.1.3 - 11	Method No. NHEERL-AED SOP 1.03.006: Chronic Echinoderm Fertilization Test

bioassay - estuarine environments

Fact Sheet # 2.1.3 - 10	Method No. NHEERL-AED SOP 1.03.005: Chronic Estuarine Survival, Growth, and Fecundity Test
Fact Sheet # 2.1.3 - 11	Method No. NHEERL-AED SOP 1.03.006: Chronic Echinoderm Fertilization Test
Fact Sheet # 2.2.3 - 12	Chronic Estuarine Amphipod Sediment Bioassay

bioassay – fish

Fact Sheet # 2.1.3 - 2	Method No. ERT SOP 2022: Acute Freshwater fish Bioassay
Fact Sheet # 2.1.3 - 6	Method No. ERT SOP 2026: Chronic Freshwater Fish Bioassay, ERT SOP 2026
Fact Sheet # 2.1.3 - 9	Method No. NHEERL-AED SOP 1.03.003: Acute Marine Fish Bioassay
Fact Sheet # 2.1.3 - 12	Method No. NHEERL-AED SOP 1.03.004: Chronic Marine Fish Bioassay

bioassay - freshwater environments

Fact Sheet # 2.1.3 - 1	Method No. ERT SOP 2024: Acute Freshwater Crustacean Bioassay: 48 Hours
Fact Sheet # 2.1.3 - 2	Method No. ERT SOP 2022: Acute Freshwater fish Bioassay
Fact Sheet # 2.1.3 - 3	Method No. ERT SOP 2027: Chronic Freshwater Algae Test
Fact Sheet # 2.1.3 – 4	Method No. ERT SOP 2025: Chronic Freshwater Crustacean Bioassay (7day)
Fact Sheet # 2.1.3 - 5	Method No. ERT SOP 2028: Chronic Freshwater Crustaceans Bioassay (10 days)
Fact Sheet # 2.1.3 - 6	Method No. ERT SOP 2026: Chronic Freshwater Fish Bioassay, ERT SOP 2026
Fact Sheet # 2.2.3 - 1	Acute Freshwater Crustacean Sediment Bioassay: Flow-through
Fact Sheet # 2.2.3 - 2	Acute Freshwater Crustacean Sediment Bioassay: In Situ Exposures
Fact Sheet # 2.2.3 - 3	Acute Freshwater Crustacean Sediment Bioassay: Static Laboratory Exposures
Fact Sheet # 2.2.3 - 4	Acute Freshwater Amphipod and Freshwater Insect Larvae Sediment Bioassay, EPA Method 100.1
Fact Sheet # 2.2.3 - 5	Chronic Freshwater Amphipod Sediment Bioassay, EPA Method 100.4
Fact Sheet # 2.2.3 - 6	Life-Cycle Freshwater Midge Sediment Bioassay, EPA Method 100.5

bioassay - marine environments

Fact Sheet # 2.1.3 - 7	Method No. NHEERL-AED SOP 1.03.001: Chronic Marine Macroalgae, Champia parvula, Sexual Reproduction test

cadmium

Fact Sheet # 2.1.1 - 11	Method No. EPA Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels
Fact Sheet # 2.1.2 - 4	EPA Method No. 1639: Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption
Fact Sheet # 2.1.2 - 6	EPA Method No. 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma — Mass Spectrometry
Fact Sheet # 2.1.2 - 5	EPA Method No. 1637: Determination of Trace Elements in Ambient Waters by Off-Line Chelation Pre-concentration and Stabilized Temperature Graphite Furnace Atomic Absorption
Fact Sheet # 2.1.2 - 7	EPA Method No. 1640: Determination of Trace Elements in Ambient Waters by On-Line Chelation Pre-concentration and Inductively Coupled Plasma-Mass Spectrometry
Fact Sheet # 2.2.2 - 2	Trace Element Quantification Techniques
Fact Sheet # 2.2.2 - 3	Method Title: Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Fact Sheet # 2.3.2 - 4	Method No. NS&T, Method Title: Trace Element Quantification Techniques
Fact Sheet # 2.3.2 - 5	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry

calcium

Fact Sheet # 2.1.2 - 34	Method No. LMMB 095: Total Hardness Titration

carbon-14

Fact Sheet # 2.3.1 - 4	Primary Productivity Using 14C: Field Procedure in the Great Lakes, LMMB 016

cesium-137

Fact Sheet # 2.2.2 - 25	Method Title: Sediment Age Dating Using Cesium-137

chemical fishing techniques

Fact Sheet # 2.3.1 - 20	Chemical Fishing

chloride

Fact Sheet # 2.1.2 - 27	Method No. ESS Method 140.4: Chloride - Automated Flow Injection Analysis
Fact Sheet # 2.1.2 - 28	Method No. ESS Method 200.5: Determination of Inorganic Anions in Water by Ion Chromatography

chlorophyll-a

Fact Sheet # 2.3.1 - 2	Chlorophyll-a Sampling Method and Preservation: Field Procedure in the Great Lakes, LMMB 015
Fact Sheet # 2.3.1 - 3	Chlorophyll-a and Phaeophytin Field Filtering Protocols
Fact Sheet # 2.3.3 - 2	Laboratory Periphyton Biomass Determination

chromium

Fact Sheet # 2.1.1 - 11	Method No. EPA Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels
Fact Sheet # 2.1.2 - 4	EPA Method No. 1639: Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption
Fact Sheet # 2.1.2 -10	EPA Method No. 1636, Method Title: Determination of Hexavalent Chromium by Ion Chromatography
Fact Sheet # 2.2.2 - 2	Trace Element Quantification Techniques

dissolved oxygen

Fact Sheet # 2.1.1 - 1	Method Title: *In Situ* sampling with the Hydrolab Datasonde3® Unit
Fact Sheet # 2.1.1 - 2	*In Situ* Dissolved Oxygen sampling with a YSI Model 58 Dissolved Oxygen Meter and probe DO meter
Fact Sheet # 2.1.1 - 9	Sample and Preservation of Water Specific Parameters

dry weight determination

Fact Sheet # 2.3.2 - 14	Determination of Percent Dry Weight for Tissues
Fact Sheet # 2.2.2 - 17	Procedures for Water Content Determination

electrofishing

Fact Sheet # 2.3.1 - 19	Electrofishing

entrapment devices

Fact Sheet # 2.3.1 - 23	Entrapment Devices

field sampling - *in situ* measurements

Fact Sheet # 2.1.1-1	*In Situ* sampling with the Hydrolab Datasonde3® Unit
Fact Sheet # 2.1.1-2	*In Situ* Dissolved Oxygen sampling with a YSI Model 58 Dissolved Oxygen Meter and probe DO meter
Fact Sheet # 2.1.1-3	*In Situ* sampling of Irradiance
Fact Sheet # 2.1.1-4	*In Situ* Transparency Sampling
Fact Sheet # 2.2.2 - 24	Field Portable X-Ray Fluorescence Spectrometry for the Determination of Elemental Concentrations in Soil and Sediment
Fact Sheet # 2.3.1 - 6	Field-based Periphyton Survey in Wadeable Streams

field sampling - discrete sample collection

Fact Sheet # 2.1.1-5	Sample Collection Procedures for Marine water
Fact Sheet # 2.1.1-6	Method No. LMMB 013: Field Sampling Using a Rosette Sampler
Fact Sheet # 2.1.1 - 7	Method No. ERT SOP #2013: Water Sample Collection with the Kemmerer Bottle and the Bacon Bomb Sampler
Fact Sheet # 2.1.1 -8	Method No. ERT SOP # 2013: Dip Sampler
Fact Sheet # 2.1.1 - 9	Sample and Preservation of Water Specific Parameters
Fact Sheet # 2.1.1 - 10	Method No. LMMB 014: Sampling of Particulate-Phase and Dissolved-Phase Organic Carbon in Great Lakes Waters
Fact Sheet # 2.1.1 - 17	LMMB 017: USGS Field Operation Plan: Tributary Monitoring
Fact Sheet # 2.1.1 - 19	Seepage Meters
Fact Sheet # 2.3.1 - 1	Phytoplankton Sample Collection and Preservation in the Great Lakes, LMMB 023t
Fact Sheet # 2.3.1 - 2	Chlorophyll-a Sampling Method and Preservation: Field Procedure in the Great Lakes, LMMB 015
Fact Sheet # 2.3.1 - 3	Chlorophyll-a and Phaeophytin Field Filtering Protocols

fish collection

Fact Sheet # 2.3.1 - 19	Electrofishing
Fact Sheet # 2.3.1 - 20	Chemical Fishing
Fact Sheet # 2.3.1 - 21	Fish Collection Using Seine Nets
Fact Sheet # 2.3.1 - 22	Entanglement Nets
Fact Sheet # 2.3.1 - 23	Entrapment Devices
Fact Sheet # 2.3.1 - 24	Pop Nets
Fact Sheet # 2.3.1 - 25	Trawls

fish community assessment

Fact Sheet # 2.3.3 - 10	Fish Bioassessment I and II

Inductively Coupled Plasma Mass Spectrometry (ICPMS)

Fact Sheet # 2.2.2 - 3	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Fact Sheet # 2.3.2 - 5	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry

iron

Fact Sheet # 2.2.2 - 2	Trace Element Quantification Techniques
Fact Sheet # 2.2.2 - 3	Method Title: Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Fact Sheet # 2.3.2 - 4	Method No. NS&T, Method Title: Trace Element Quantification Techniques
Fact Sheet # 2.3.2 - 5	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry

lead

Fact Sheet # 2.1.1 - 11	Method No. EPA Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels
Fact Sheet # 2.1.2 - 6	EPA Method No. 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma — Mass Spectrometry
Fact Sheet # 2.1.2 - 5	EPA Method No. 1637: Determination of Trace Elements in Ambient Waters by Off-Line Chelation Pre-concentration and Stabilized Temperature Graphite Furnace Atomic Absorption
Fact Sheet # 2.1.2 - 7	EPA Method No. 1640: Determination of Trace Elements in Ambient Waters by On-Line Chelation Pre-concentration and Inductively Coupled Plasma-Mass Spectrometry
Fact Sheet # 2.2.2 - 2	Trace Element Quantification Techniques
Fact Sheet # 2.2.2 - 3	Method Title: Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Fact Sheet # 2.3.2 - 4	Method No. NS&T, Method Title: Trace Element Quantification Techniques
Fact Sheet # 2.3.2 - 5	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry

lead-210

Fact Sheet # 2.2.2-15	Method No. LMMB 084, Method Title: Determination of the Activity of Lead-210 in Sediments and Soils

light measurements

Fact Sheet # 2.1.1-3	*In Situ* sampling of Irradiance

macroinvertebrate – analysis

Fact Sheet # 2.3.3 - 3	Laboratory Analysis of Benthic Macroinvertebrates in Wadeable Streams
Fact Sheet # 2.3.3 - 4	Laboratory Analysis of Water Column Organisms

macroinvertebrate - sample collection

Fact Sheet # 2.2.1 - 1	Grab Sampling
Fact Sheet # 2.2.1 - 2	Core Samplers
Fact Sheet # 2.2.1 - 3	Hand Collection
Fact Sheet # 2.2.1 - 4	Hand collection at depth with SCUBA
Fact Sheet # 2.3.1 - 9	Artificial Substrate Samplers of Macroinvertebrates in Wadeable Streams
Fact Sheet # 2.3.1 - 10	Algae and Macroinvertebrate Sampling with Frames
Fact Sheet # 2.3.1 - 11	Benthic Organism Collection from a Marine Environment, NHEERL-AED SOP 1.02.001

Fact Sheet # 2.1.2 - 9	EPA Method No. 1632, Revision A: Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry
Fact Sheet # 2.1.2 - 10	EPA Method No. 1636: Determination of Hexavalent Chromium by Ion Chromatography
Fact Sheet # 2.2.2 - 2	Trace Element Quantification Techniques
Fact Sheet # 2.3.2 - 1	Sample Preparation for Metal Contaminants in Tissue
Fact Sheet # 2.3.2 - 4	Trace Element Quantification Techniques
Fact Sheet # 2.3.2 - 5	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Fact Sheet # 2.3.2 - 6	Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry, EPA Method 1632, Revision A

metals toxicity

Fact Sheet # 2.1.2 - 4	EPA Method No. 1639: Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption

microbiological testing

Fact Sheet # 2.1.2 - 5	EPA Method No. 1637: Determination of Trace Elements in Ambient Waters by Off-Line Chelation Pre-concentration and Stabilized Temperature Graphite Furnace Atomic Absorption

microwave extraction

Fact Sheet # 2.3.2 - 16	Microwave Extraction of Marine Tissue for Semivolatile Organic Analytes, AED LOP 2.03.030, Revision 0

mussels

Fact Sheet # 2.1.1 - 20	Caged Bivalve Deployment
Fact Sheet # 2.3.1 - 18	Mussel Collection Using Brails

mutagenicity testing

Fact Sheet # 2.2.3 - 14	Ames Mutagenicity Assay
Fact Sheet # 2.2.3 - 15	Mutatox Genotoxicity Assay
Fact Sheet # 2.2.3 - 16	V79 Sister Chromatid Exchange Assay, NHEERL-AED SOP 1.03.012

net tow surveys

Fact Sheet # 2.3.1 - 5	Zooplankton Sample Collection and Preservation in the Great Lakes, LMMB 024
Fact Sheet # 2.3.1 - 13	Benthic Macroinvertebrate Protocols in a Wadeable Stream: Multi-habitat Approach: D-Frame Dip Net
Fact Sheet # 2.3.1 - 16	Macroinvertebrate Drift Nets in a Wadeable Stream
Fact Sheet # 2.3.1 - 21	Fish Collection Using Seine Nets
Fact Sheet # 2.3.1 - 22	Entanglement Nets
Fact Sheet # 2.3.1 - 24	Pop Nets

nickel

Fact Sheet # 2.1.1 - 11	Method No. EPA Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels
Fact Sheet # 2.1.2 - 4	EPA Method No. 1639: Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption
Fact Sheet # 2.1.2 - 6	EPA Method No. 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma — Mass Spectrometry
Fact Sheet # 2.1.2 - 7	EPA Method No. 1640: Determination of Trace Elements in Ambient Waters by On-Line Chelation Pre-concentration and Inductively Coupled Plasma-Mass Spectrometry
Fact Sheet # 2.2.2 - 2	Trace Element Quantification Techniques
Fact Sheet # 2.2.2 - 3	Method Title: Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Fact Sheet # 2.3.2 - 4	Method No. NS&T, Method Title: Trace Element Quantification Techniques
Fact Sheet # 2.3.2 - 5	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry

nitrogen determination

Fact Sheet # 2.1.2 - 20	Method No. ESS Method 220.3: Ammonia Nitrogen and Nitrate+Nitrite Nitrogen, Automated Flow Injection Analysis Method
Fact Sheet # 2.1.2 - 21	Method No. ESS Method 230.1: Total Phosphorus and Total Kjeldahl Nitrogen, Semi-Automated Method
Fact Sheet # 2.1.2 - 28	Method No. ESS Method 200.5: Determination of Inorganic Anions in Water by Ion Chromatography

organic analysis methods

Fact Sheet # 2.1.1 - 9	Sample and Preservation of Water Specific Parameters
Fact Sheet # 2.1.1 - 10	Method No. LMMB 014: Sampling of Particulate-Phase and Dissolved-Phase Organic Carbon in Great Lakes Waters
Fact Sheet # 2.1.2 - 11	EPA Method No. 1624b: Volatile Organic Compounds by Isotope Dilution GC/MS
Fact Sheet # 2.1.2 - 13	EPA Method No. 1625: Semi-volatile Organic Compounds by Isotope Dilution GC/MS
Fact Sheet # 2.1.2 - 14	Quantitative Determination of Polynuclear Aromatic Hydrocarbons by Gas Chromatography/Mass Spectrometry (GC/MS) - Selected Ion Monitoring (SIM) Mode
Fact Sheet # 2.1.2 - 15	Method No. LMMB 041: Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection
Fact Sheet # 2.1.2 - 16	Method No. LMMB: PCBs and Pesticides in Surface Water by XAD-2 Resin Extraction
Fact Sheet # 2.1.2 - 18	EPA Method No. 1668: Toxic Polychlorinated Biphenyls by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry
Fact Sheet # 2.1.2 - 19	EPA Method No. 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS
Fact Sheet # 2.3.2 - 7	Extraction and Lipid Separation of Fish Samples for Contaminant Analysis and Lipid Determination, LMMB 043

organotins

Fact Sheet # 2.2.2 - 13	Butyltin in Sediments

Fact Sheet # 2.3.2 - 7	Extraction and Lipid Separation of Fish Samples for Contaminant Analysis and Lipid Determination, LMMB 043
Fact Sheet # 2.3.2 - 8	Purification of Biological Tissue Samples by Gel Permeation Chromatography of Organic Analyses
Fact Sheet # 2.3.2 - 10	Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection, LMMB 041
Fact Sheet # 2.3.2 - 12	Toxic Polychlorinated Biphenyls by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry, EPA Method 1668
Fact Sheet # 2.3.2 - 13	Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, EPA Method 1668 Revision A

percent dry weight

Fact Sheet # 2.3.2 - 14	Determination of Percent Dry Weight for Tissues

percent lipid

Fact Sheet # 2.3.2 - 15	Determination of Percent Lipid in Tissue

percent moisture

Fact Sheet # 2.3.2 - 14	Determination of Percent Dry Weight for Tissues

periphyton

Fact Sheet # 2.3.1 - 6	Field-based Periphyton Survey in Wadeable Streams
Fact Sheet # 2.3.1 - 7	Laboratory-based Periphyton Survey: Single Habitat Sampling in Wadeable Streams
Fact Sheet # 2.3.1 - 8	Laboratory-based Rapid Periphyton Survey: Multi-habitat Sampling in Wadeable Streams
Fact Sheet # 2.3.1 - 9	Artificial Substrate Samplers of Macroinvertebrates in Wadeable Streams
Fact Sheet # 2.3.3 - 1	Laboratory Identification, Enumeration and Biomass Measurements of Periphyton in Wadeable Streams
Fact Sheet # 2.3.3 - 2	Laboratory Periphyton Biomass Determination

permeability

Fact Sheet # 2.2.2 - 20	Standard Test Method for Permeability of Granular Soils (Constant Head), ASTM Method D2434

pesticide analysis methods

Fact Sheet # 2.1.2 - 16	Method No. LMMB: PCBs and Pesticides in Surface Water by XAD-2 Resin Extraction
Fact Sheet # 2.2.2 - 8	Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection, LMMB 041
Fact Sheet # 2.3.2 - 8	Purification of Biological Tissue Samples by Gel Permeation Chromatography of Organic Analyses
Fact Sheet # 2.3.2 - 10	Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection, LMMB 041

pH

Fact Sheet # 2.1.2 - 29	Method No. LMMB 092: Standard Operating Procedure for Electrometric pH

phosphorus

Fact Sheet # 2.1.2 - 21	Method No. ESS Method 230.1: Total Phosphorus and Total Kjeldahl Nitrogen, Semi-Automated Method
Fact Sheet # 2.1.2 - 22	Method No. ESS Method 310.2, LMMB 064: Phosphorus, Total, Low Level (Persulfate Digestion)
Fact Sheet # 2.1.2 - 23	Method No. ESS Method 310.1, LMMB 063: Ortho-Phosphorus, Dissolved Automated, Ascorbic Acid

photographic surveys

Fact Sheet # 2.3.1 - 14	Photographic Habitat Documentation of the Benthic Community
Fact Sheet # 2.3.1 - 15	Sediment Profile Camera

phytoplankton sampling

Fact Sheet # 2.3.1 - 1	Phytoplankton Sample Collection and Preservation in the Great Lakes, LMMB 023t

plasticity index

Fact Sheet # 2.2.2 - 23	Standard Test Method for Liquid Limit, Plastic Limit, and Plasticity Index of Soils, ASTM Method D4318

polychlorinated biphenyls (see PCB analysis methods)

polycyclic aromatic hydrocarbons (see PAH analysis methods)

pore water

Fact Sheet # 2.1.1 - 13	*In Situ* Peepers
Fact Sheet # 2.1.1 - 14	Suction samplers
Fact Sheet # 2.2.1 - 10	Method No. ASTM E 1391-94: Pore Water Extraction through Centrifugation
Fact Sheet # 2.2.1 - 11	Method No. ASTM E 1391-94: Pore Water Extraction from Sediments through Squeezing
Fact Sheet # 2.2.1 - 12	Method No. ASTM E 1391-94: Pore water extraction from sediment from Vacuum Filtration, Gas Pressurization, or Displacement

primary productivity

Fact Sheet # 2.3.1 - 4	Primary Productivity Using 14C: Field Procedure in the Great Lakes, LMMB 016

radioisotopes

Fact Sheet # 2.2.2 - 15	Determination of the Activity of Lead-210 in Sediments and Soils, LMMB 084
Fact Sheet # 2.2.2 - 25	Sediment Age Dating Using Cesium-137
Fact Sheet # 2.2.2 - 26	Beryllium-7 as a Tracer of Short Term Sediment Deposition
Fact Sheet # 2.3.1 - 4	Primary Productivity Using 14C: Field Procedure in the Great Lakes, LMMB 016

respiration rate

Fact Sheet # 2.1.3 - 15	Method No. NHEERL-AED SOP 1.03.013: Growth and Scope for Growth Measurements with Mytilus edulis

scope for growth (SFG) index

Fact Sheet # 2.1.3 - 15	Method No. NHEERL-AED SOP 1.03.013: Growth and Scope for Growth Measurements with Mytilus edulis

SCUBA

Fact Sheet # 2.2.1 - 4	Hand collection at depth with SCUBA
Fact Sheet # 2.3.1 - 14	Photographic Habitat Documentation of the Benthic Community

sea floor mapping

Fact Sheet # 2.2.1 - 13	Method No. DRP-2-03: Acoustic Sub-bottom Profiling Systems
Fact Sheet # 2.2.1 - 14	Method No. EEDP-01-10: Side Scan Sonar

sediment consolidation

Fact Sheet # 2.2.1 - 15	Method No. DRP-2-3: Settlement Phases
Fact Sheet # 2.2.2 - 21	Standard Test Method for One-Dimensional Consolidation Properties of Soil, ASTM Method D2435

sediment cores

Fact Sheet # 2.2.1 - 2	Core Samplers
Fact Sheet # 2.2.1 - 6	Russian Peat Borer
Fact Sheet # 2.2.1 - 7	Split Core Sampler for Submerged Sediments

sediment dating

Fact Sheet # 2.2.2 - 15	Determination of the Activity of Lead-210 in Sediments and Soils, LMMB 084
Fact Sheet # 2.2.2-25	Sediment Age Dating Using Cesium-137
Fact Sheet # 2.2.2-26	Beryllium-7 as a Tracer of Short Term Sediment Deposition

sediment grain size

Fact Sheet # 2.2.2 - 16	Sediment Grain Size Analysis, NHEERL-AED SOP 1.01.005

sediment flux

Fact Sheet # 2.1.1 - 19	Seepage Meters
Fact Sheet # 2.2.1 - 4	Hand collection at depth with SCUBA

sediment sampling

Fact Sheet # 2.2.1 - 1	Grab Sampling
Fact Sheet # 2.2.1 - 2	Core Samplers
Fact Sheet # 2.2.1 - 3	Hand Collection
Fact Sheet # 2.2.1 - 4	Hand collection at depth with SCUBA
Fact Sheet # 2.2.1 - 5	Sediment traps
Fact Sheet # 2.2.1 - 6	Russian Peat Borer
Fact Sheet # 2.2.1 - 7	Split Core Sampler for Submerged Sediments
Fact Sheet # 2.3.1 - 11	Benthic Organism Collection from a Marine Environment, NHEERL-AED SOP 1.02.001
Fact Sheet # 2.3.1 - 12	Benthic Macroinvertebrate Protocols in a Wadeable Stream: Single Habitat Approach, 1-Meter Kick Net
Fact Sheet # 2.3.1 - 13	Benthic Macroinvertebrate Protocols in a Wadeable Stream: Multi-habitat Approach: D-Frame Dip Net

sediment toxicity

Fact Sheet # 2.2.3 - 1	Acute Freshwater Crustacean Sediment Bioassay: Flow-through
Fact Sheet # 2.2.3 - 3	Acute Freshwater Crustacean Sediment Bioassay: Static Laboratory Exposures
Fact Sheet # 2.2.3 - 4	Acute Freshwater Amphipod and Freshwater Insect Larvae Sediment Bioassay, EPA Method 100.1
Fact Sheet # 2.2.3 - 5	Chronic Freshwater Amphipod Sediment Bioassay, EPA Method 100.4
Fact Sheet # 2.2.3 - 6	Life-Cycle Freshwater Midge Sediment Bioassay, EPA Method 100.5
Fact Sheet # 2.2.3 - 7	Acute Larval Bivalve Sediment Bioassay

Fact Sheet # 2.2.3 - 8	Acute Echinoderm Sediment Bioassay
Fact Sheet # 2.2.3 - 9	Acute Marine Crustacean Sediment Bioassay
Fact Sheet # 2.2.3 - 10	Acute Marine Amphipod Crustacean Sediment Bioassay, EPA Method 100.4
Fact Sheet # 2.2.3 - 11	Acute Marine Polychaete Sediment Bioassay, ASTM Method E1611-00
Fact Sheet # 2.2.3 - 12	Chronic Estuarine Amphipod Sediment Bioassay
Fact Sheet # 2.2.3 - 13	Chronic Marine Polychaete Sediment Bioassay, ASTM Method E1611-00
Fact Sheet # 2.2.3 - 14	Ames Mutagenicity Assay
Fact Sheet # 2.2.3 - 15	Mutatox Genotoxicity Assay
Fact Sheet # 2.2.3 - 16	V79 Sister Chromatid Exchange Assay, NHEERL-AED SOP 1.03.012
Fact Sheet # 2.2.3 - 17	Bioaccumulation Test for Marine, Estuarine, and Freshwater Sediments, EPA Method 100.3

sediment water content

Fact Sheet # 2.2.2 - 17	Procedures for Water Content Determination

selected ion monitoring (SIM)

Fact Sheet # 2.2.2 - 7	Quantitative Determination of Polynuclear Aromatic Hydrocarbons by Gas Chromatography/Mass Spectrometry (GC/MS)--Selected Ion Monitoring (SIM) Mode
Fact Sheet # 2.3.2 - 9	Quantitative Determination of Polynuclear Aromatic Hydrocarbons by Gas Chromatography/Mass Spectrometry (GC/MS)--Selected Ion Monitoring (SIM) Mode

selenium

Fact Sheet # 2.1.1 - 11	Method No. EPA Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels
Fact Sheet # 2.1.2 - 4	EPA Method No. 1639: Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption
Fact Sheet # 2.1.2 - 6	EPA Method No. 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma — Mass Spectrometry
Fact Sheet # 2.2.2 - 2	Trace Element Quantification Techniques
Fact Sheet # 2.2.2 - 3	Method Title: Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Fact Sheet # 2.3.2 - 4	Method No. NS&T, Method Title: Trace Element Quantification Techniques
Fact Sheet # 2.3.2 - 5	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry

semi-volatile organic compounds

Fact Sheet # 2.1.2 - 13	EPA Method No. 1625: Semi-volatile Organic Compounds by Isotope Dilution GC/MS
Fact Sheet # 2.2.2 - 6	Extraction and Clean-up of Sediments for Semi-volatile Organics Following the Internal Standard Method, LMMB 040
Fact Sheet # 2.3.2 - 16	Microwave Extraction of Marine Tissue for Semivolatile Organic Analytes, AED LOP 2.03.030, Revision 0

settlement plates

Fact Sheet # 2.2.1 - 15	Method No. DRP-2-3: Settlement Phases

settling particulate matter

Fact Sheet # 2.2.1 - 5	Sediment traps

shear strength

Fact Sheet # 2.2.2 - 18	Standard Test Method for Field Vane Shear Test in Cohesive Soil, ASTM Method D2573

side scan sonar

Fact Sheet # 2.2.1 - 14	Method No. EEDP-01-10: Side Scan Sonar

silt

Fact Sheet # 2.2.2 – 16	Sediment Grain Size Analysis, NHEERL-AED SOP 1.01.005

silicon

Fact Sheet # 2.2.2 - 3	Method Title: Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry

silver

Fact Sheet # 2.1.1 - 11	Method No. EPA Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels
Fact Sheet # 2.1.2 - 6	EPA Method No. 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma — Mass Spectrometry
Fact Sheet # 2.2.2 - 3	Method Title: Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Fact Sheet # 2.3.2 - 4	Method No. NS&T, Method Title: Trace Element Quantification Techniques

simultaneously extracted metals (SEM)

Fact Sheet # 2.2.2 - 4	Determination of Acid Volatile Sulfide and Selected Simultaneously Extractable Metals in Sediment

soil classification

Fact Sheet # 2.2.2 - 18	Standard Test Method for Field Vane Shear Test in Cohesive Soil, ASTM Method D2573
Fact Sheet # 2.2.2 - 19	Standard Test Method for Specific Gravity of Soil Solids by Water Pycnometer, ASTM Method D854
Fact Sheet # 2.2.2 - 20	Standard Test Method for Permeability of Granular Soils (Constant Head), ASTM Method D2434
Fact Sheet # 2.2.2 - 21	Standard Test Method for One-Dimensional Consolidation Properties of Soil, ASTM Method D2435
Fact Sheet # 2.2.2 - 22	Standard Test Method for Classification of Soils for Engineering Purposes (Unified Soil Classification System), ASTM Method D2487
Fact Sheet # 2.2.2 - 23	Standard Test Method for Liquid Limit, Plastic Limit, and Plasticity Index of Soils, ASTM Method D4318

specific gravity

Fact Sheet # 2.2.2 - 19	Standard Test Method for Specific Gravity of Soil Solids by Water Pycnometer, ASTM Method D854

stream characterization

Fact Sheet # 2.1.1 - 15	Physical Characterization of a stream
Fact Sheet # 2.1.1 - 16	Visual based habitat assessment
Fact Sheet # 2.1.1 - 17	LMMB 017: USGS Field Operation Plan: Tributary Monitoring

sub-bottom profiling

Fact Sheet # 2.2.1 - 13	Method No. DRP-2-03: Acoustic Sub-bottom Profiling Systems

sulfate

Fact Sheet # 2.1.2 - 28	Method No. ESS Method 200.5: Determination of Inorganic Anions in Water by Ion Chromatography

thallium

Fact Sheet # 2.1.1 - 11	Method No. EPA Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels
Fact Sheet # 2.1.2 - 6	EPA Method No. 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma — Mass Spectrometry

tin

Fact Sheet # 2.2.2 - 2	Trace Element Quantification Techniques
Fact Sheet # 2.2.2 - 3	Method Title: Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Fact Sheet # 2.3.2 - 4	Method No. NS&T, Method Title: Trace Element Quantification Techniques

total organic carbon (TOC)

Fact Sheet # 2.1.2 - 24	Standard Method No. 5310: Total Organic Carbon
Fact Sheet # 2.2.2 - 14	Procedures for Sediment Total Organic Carbon (TOC) Determination

total suspended solids (TSS)

Fact Sheet # 2.1.2 - 32	Method No. LMMB 090: Standard Operating Procedure for GLNPO Turbidity: Nephelometeric Method
Fact Sheet # 2.1.2 - 33	Method No. LMMB 065: ESS Method 340.2: Total Suspended Solids, Mass Balance (Dried at 103-105EC) Volatile Suspended Solids (Ignited at 550EC)

toxicity testing

Fact Sheet # 2.1.3 - 1	Method No. ERT SOP 2024: Acute Freshwater Crustacean Bioassay: 48 Hours
Fact Sheet # 2.1.3 - 2	Method No. ERT SOP 2022: Acute Freshwater fish Bioassay
Fact Sheet # 2.1.3 - 3	Method No. ERT SOP 2027: Chronic Freshwater Algae Test
Fact Sheet # 2.1.3 - 4	Method No. ERT SOP 2025: Chronic Freshwater Crustacean Bioassay (7day)
Fact Sheet # 2.1.3 - 5	Method No. ERT SOP 2028: Chronic Freshwater Crustaceans Bioassay (10 days)
Fact Sheet # 2.1.3 - 6	Method No. ERT SOP 2026: Chronic Freshwater Fish Bioassay, ERT SOP 2026
Fact Sheet # 2.1.3 - 7	Method No. NHEERL-AED SOP 1.03.001: Chronic Marine Macroalgae, *Champia parvula*, Sexual Reproduction test
Fact Sheet # 2.1.3 - 8	Method No. NHEERL-AED SOP 1.03.003: Acute Marine Crustacean Bioassay
Fact Sheet # 2.1.3 - 9	Method No. NHEERL-AED SOP 1.03.003: Acute Marine Fish Bioassay
Fact Sheet # 2.1.3 - 10	Method No. NHEERL-AED SOP 1.03.005: Chronic Estuarine Survival, Growth, and Fecundity Test
Fact Sheet # 2.1.3 - 11	Method No. NHEERL-AED SOP 1.03.006: Chronic Echinoderm Fertilization Test
Fact Sheet # 2.1.3 - 12	Method No. NHEERL-AED SOP 1.03.004: Chronic Marine Fish Bioassay
Fact Sheet # 2.1.3 - 13	Toxicity Evaluations of Photoinduction of Polycyclic Aromatic Hydrocarbons (PAH): In Situ Analysis
Fact Sheet # 2.1.3 - 14	Toxicity Evaluations of Photoinduction of Polycyclic Aromatic Hydrocarbons: Laboratory Analysis of Storm water

Fact Sheet # 2.1.3 - 16	Method No. NHEERL-AED SOP 1.03.009: Microtox® tests, NHEERL-AED 1.03.009
Fact Sheet # 2.2.3 - 1	Acute Freshwater Crustacean Sediment Bioassay: Flow-through
Fact Sheet # 2.2.3 - 2	Acute Freshwater Crustacean Sediment Bioassay: In Situ Exposures

toxicology testing - sample prep

Fact Sheet # 2.2.1 - 8	Sediment Processing for Chemistry and Toxicity Testing
Fact Sheet # 2.2.1 - 9	Sediment Processing for Elutriate Toxicity tests
Fact Sheet # 2.2.1 - 10	Method No. ASTM E 1391-94: Pore Water Extraction through Centrifugation
Fact Sheet # 2.2.1 - 11	Method No. ASTM E 1391-94: Pore Water Extraction from Sediments through Squeezing
Fact Sheet # 2.2.1 - 12	Method No. ASTM E 1391-94: Pore water extraction from sediment from Vacuum Filtration, Gas Pressurization, or Displacement

trace metals analysis

Fact Sheet # 2.1.2 - 1	EPA Method No. 245.7: Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry
Fact Sheet # 2.1.2 - 2	EPA Method No. 1631, Revision B: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry
Fact Sheet # 2.1.2 - 3	EPA Method No. 1630: Methyl mercury in water by distillation, Aqueous Ethylation, Purge and Trap, and CVAFS
Fact Sheet # 2.1.2 - 4	EPA Method No. 1639: Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption
Fact Sheet # 2.1.2 - 5	EPA Method No. 1637: Determination of Trace Elements in Ambient Waters by Off-Line Chelation Pre-concentration and Stabilized Temperature Graphite Furnace Atomic Absorption
Fact Sheet # 2.1.2 - 6	EPA Method No. 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma — Mass Spectrometry
Fact Sheet # 2.1.2 - 7	EPA Method No. 1640: Determination of Trace Elements in Ambient Waters by On-Line Chelation Pre-concentration and Inductively Coupled Plasma-Mass Spectrometry
Fact Sheet # 2.2.2 - 2	Trace Element Quantification Techniques
Fact Sheet # 2.2.2 - 3	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry
Fact Sheet # 2.3.2 - 4	Trace Element Quantification Techniques
Fact Sheet # 2.3.2 - 5	Analysis of Marine Sediment and Bivalve Tissue by X-Ray Fluorescence, Atomic Absorption and Inductively Coupled Plasma Mass Spectrometry

transparency

Fact Sheet # 2.1.1-4	*In Situ* Transparency Sampling

trawling

Fact Sheet # 2.3.1 - 25	Trawls

turbidity

Fact Sheet # 2.1.2 - 32	Method No. LMMB 090: Standard Operating Procedure for GLNPO Turbidity: Nephelometeric Method

volatile organic compounds (VOC)

Fact Sheet # 2.1.2 - 12	Method No. OERR SOP #2109: Photovac GC Analysis for Soil, Water, and Air/Soil Gas
Fact Sheet # 2.2.2 - 5	Photovac GC Analysis for Soil, Water, and Air/Soil Gas, OSWER SOP# 2109

water column characterization

United States
Environmental Protection
Agency

National Risk Management
Research Laboratory
Cincinnati, OH 45268

Official Business
Penalty for Private Use
$300

EPA/600/R-03/108
April 2004

Please make all necessary changes on the below label, detach or copy, and return to the address in the upper left-hand corner.

If you do not wish to receive these reports CHECK HERE ☐; detach, or copy this cover, and return to the address in the upper left-hand corner.

Presorted Standard
Postate & Fees Paid
EPA
Permit No. G-35