FINAL REPORT
TC-3991-04

Puget Sound Estuary Program

INTRODUCTION

Prepared by:

TETRA TECH, INC.

Prepared for:

U.S. ENVIRONMENTAL PROTECTION AGENCY
Region 10 - Office of Puget Sound
Seattle, WA

and

U.S. Army Corps of Engineers
Seattle District
Seattle, WA

March, 1986

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ACKNOWLEDGEMENTS

This chapter was prepared by Tetra Tech, Inc., under the direction of Dr. Scott Becker, for the U.S. Environmental Protection Agency in partial fulfillment of Contract No. 68-03-1977. Dr. Thomas Ginn of Tetra Tech was the Program Manager. Mr. John Underwood and Dr. John Armstrong of U.S. EPA were the Project Officers.

PURPOSE

Environmental variables in Puget Sound are measured by a wide variety of organizations, including government agencies, universities, and private institutions. However, comparisons of results of different studies frequently are limited because different methods are used to measure the same variable(s). The ability to compare data among different studies is highly desirable for developing a comprehensive management strategy for the Sound.

This document (i.e., notebook) presents recommended protocols for measuring selected environmental variables in Puget Sound. The objective is to encourage most investigators conducting studies such as monitoring programs, baseline surveys, and intensive investigations to use equivalent methods whenever possible. If this objective is achieved, most data from future sampling programs should be comparable among studies. It is recognized that alternative methods exist for many of the variables considered in this document and that those methods may produce data of equal or better quality than do the recommended methods. However, the criterion that data should be comparable limited the range of methods recommended in this document. It is also recognized that future research or other circumstances may require modification or replacement of one or more of the recommended methods. The loose-leaf format of this document was selected specifically to allow such changes to be made.

The recommendations in this document pertain primarily to the methodological specifications required to measure the selected environmental variables. Recommendations for study design and data analysis generally were not included because those considerations vary widely depending upon the objectives of individual studies. As mentioned previously, the goal of this document is to ensure that comparable data are generated by different studies. This does not necessarily require that all studies have the same initial design, nor that all data are analyzed in the same manner after being generated. It is recommended, however, that sample collection and analysis specifications of study designs be similar enough to ensure that comparable data are produced whenever possible.

As an action separate from the preparation of this document, several of the recommended protocols will be required for use in government regulatory permit programs. For example, the Puget Sound Dredged Disposal Analysis (PSSDA) intends to specify several of the recommended protocols as requirements when conducting dredged material regulatory testing and disposal site assessments. Use of such standardized procedures is essential for making comparisons to regulatory standards and reference conditions.

SCOPE

A meeting was convened by U.S. EPA on 31 May, 1985 (Table 1) to determine the priority of variables for protocol development or documentation. Variables were ranked on the basis of three major criteria:

1. The frequency with which each variable has been measured in a variety of studies (e.g., monitoring programs, baseline surveys, intensive investigations) throughout Puget Sound

2. The importance of each variable for making decisions related to environmental problems in the Sound

3. The degree to which a variety of methods has been used to measure each variable.

Using the criteria listed above, eight groups of variables were identified as having the highest priority for protocol development or documentation. They include:

- Station positioning considerations
- Conventional sediment variables
- Concentrations of organic compounds in sediment and tissue
- Concentrations of metals in sediment and tissue
- Benthic infaunal variables
- Sediment bioassays
- Pathological conditions in fish livers
- Microbiological indicators.

Recommended protocols for all of these variables are presented in later sections of this document. In addition to these eight groups of variables, a number of others were considered appropriate for protocol development. The loose-leaf format of this document will allow additional protocols to be included in the future.

In addition to the recommended protocols for each group of variables, a section on general quality assurance/quality control (QA/QC) procedures is included in this document. That section identifies the major QA/QC concerns that should be addressed when collecting and analyzing environmental samples from Puget Sound.

TABLE 1. PARTICIPANTS OF THE VARIABLE-RANKING WORKSHOP HELD ON 31 MAY, 1985

Name	Organization
John Armstrong	U.S. EPA
Bob Barrick	Tetra Tech, Inc.
Scott Becker[a]	Tetra Tech, Inc.
Joe Blazevich	U.S. EPA
Don Brown	NOAA
Joe Cummins	U.S. EPA
Ray Dalseg	Metro
Bob Dexter	EVS Consultants
Burt Hamner	U.S. COE
Ed Long	NOAA
Dave Mitchell	Metro
Paulette Murphy	NOAA
Jeff Osborn	Parametrix, Inc.
Jerry Stober	Univ. of Wash.
Rich Tomlinson	Metro
Jack Word	Evans Hamilton, Inc.

[a] Moderator.

APPROACH

The recommended protocols for each group of variables were developed by convening a workshop comprised of representatives from most organizations that routinely measure or use the variables of concern in Puget Sound. The objective of each workshop was to evaluate various methods and, if possible, agree upon which methods should be used in the future. Consideration was given to providing data that will be comparable with the historical database. Prior to each workshop, the methods used historically in Puget Sound were evaluated and specific items requiring standardization were identified. Additional considerations for developing the various recommended protocols included data quality needs, cost, and availability of equipment and expertise.

Each workshop focused on defining acceptable methods and determining which of those methods would provide comparable data. If several acceptable methods did not provide comparable data, the workshop participants were asked to select only one for future use. As expected, a full consensus rarely was achieved. However, in many cases, the majority of participants clearly favored a single method. In other instances, the participants were relatively evenly divided between recommending two or more methods.

After each workshop, draft protocols were developed. As much as possible, recommendations of each protocol were based on the majority viewpoint of the workshop participants. In some cases, a single recommendation could not be given for a particular specification because no agreement was reached at the workshop. In such instances, various specifications used by different Puget Sound investigators were simply described.

Draft protocols were mailed to all workshop participants and other interested parties for written review. Following this review, comments made by several reviewers were incorporated into the protocols. Most major comments made by single reviewers were resolved with each respective reviewer. After all written reviews were addressed, protocols were finalized and included as a chapter of this document.

FORMAT

Each protocol in this document is designed to stand alone. However, in many studies several related variables are measured simultaneously. In such cases, the protocol for one variable may require some modification to be consistent with that of a second variable. For example, collection of sediment subsamples for analysis of conventional variables (e.g., particle size, total volatile solids, total organic carbon) normally does not require collection equipment to be washed with special solvents. However, if sediment subsamples also will be collected from the same sample for analysis of organic compounds, collection equipment for the conventionals subsample must be washed with the same solvent specified for collection of the organics subsample to avoid contaminating the latter sediment. For studies considering multiple variables, it is therefore recommended that the protocols for all relevant variables be reviewed carefully before sampling begins, to ensure that all appropriate modifications are made.

The formats for most protocols are similar to facilitate use of the entire document. The following major sections are presented for most protocols:

- Use and limitations - describes what a variable measures and major limitations to the use of the variable

- Field procedures - describes container type, special cleaning procedures, collection techniques, sample quantity, preservation technique, storage conditions, and maximum holding time

- Laboratory procedures - describes analytical procedures (or provides citations), laboratory equipment, sources of error, and QA/QC specifications

- Data reporting requirements - describes the kinds of data that the analytical laboratory should report and the units in which the data should be reported.

CAVEATS

Several notes of caution require emphasis before the protocols in this document are presented. First, these protocols were developed solely to promote the collection of comparable data in Puget Sound. A variety of other methods may exist that produce data of equal or better quality than the recommended protocols. However, the criterion that data should be comparable limited the range of methods recommended in this document.

A second caveat is that rarely was a full consensus reached with respect to any aspect of any protocol. Therefore, it should not be construed that all individuals, agencies, and institutions that participated in this effort agreed with all of the final products. The recommended protocols are simply a best effort to represent the majority viewpoints of the many individuals from diverse backgrounds that attended the workshops or commented on the draft protocols.

A third caveat is that this document is intended to be dynamic. The loose-leaf format was selected specifically for this reason. Modifications or additions to the protocols can therefore be made in the future if needs or viewpoints change, or if methodological refinements or improvements are made.

GENERAL QA/QC

FINAL REPORT
TC-3991-04

Puget Sound Estuary Program

GENERAL QA/QC CONSIDERATIONS FOR COLLECTING ENVIRONMENTAL SAMPLES IN PUGET SOUND

Prepared by:

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Prepared for:

U.S. ENVIRONMENTAL PROTECTION AGENCY
Region 10 - Office of Puget Sound
Seattle, WA

March, 1986

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ACKNOWLEDGMENTS

This chapter was prepared originally by Tetra Tech, Inc., for the Marine Operations Division, Office of Marine and Estuarine Protection, U.S. EPA, Washington, DC as part of U.S. EPA Contract No. 68-01-6938, Allison J. Duryee, Project Officer. It was prepared under the direction of Dr. Thomas Ginn (Program Manager) and Dr.Scott Becker (Project Manager) of Tetra Tech.

INTRODUCTION

Quality assurance/quality control (QA/QC) procedures are necessary to ensure that environmental data achieve an acceptable level of quality and that the level of quality attained is documented adequately. Detailed QA/QC procedures for the environmental variables considered in this document are specified in each respective recommended protocol. This section describes many of the more general QA/QC procedures that should be considered when collecting and analyzing environmental samples. These procedures are termed general because they pertain to samples collected for most kinds of environmental variables. They include procedures that should be followed when samples are collected in the field, shipped to laboratories, and stored or distributed within laboratories.

No workshop was held to evaluate the general QA/QC procedures for use in Puget Sound. However, they have been reviewed for use in U.S. EPA's 301(h) marine monitoring programs by U.S. EPA's Office of Research and Development (Narragansett, RI and Newport, OR), as well as by representatives from U.S. EPA Regions IX and X.

SAMPLING PREPARATION

The chief scientist or a designee should thoroughly review the field survey plan (including QA/QC criteria) before each cruise. The plan should be checked for completeness and clarity of objectives. A complete plan should contain the following major elements:

- Identification of scientific party and the responsibilities of each member
- Statement and prioritization of study objectives
- Description of survey area, including background information and station locations
- Identification of variables to be measured and corresponding required containers and preservatives
- Identification of all sample splits or performance samples to be submitted with the survey samples
- Brief description of sampling methods, including station positioning technique, sampling devices, replication, and any special considerations
- Detailed cruise schedule, including time, date, and location of embarkation and debarkation
- Storage and shipping procedures
- Identification of onshore laboratories to which samples should be shipped after cruise completion
- Survey vessel requirements (e.g., size, laboratory needs, sample storage needs)
- Location and availability of an alternate survey vessel
- All special equipment needed for the survey (e.g., camera, nets, communication devices).

Study objectives and their prioritization should be understood by all members of the scientific party. This will ensure that if modifications of the survey plan become necessary in the field, their impact on the overall goals of the cruise can be evaluated adequately. After the sampling plan has been reviewed, contingency plans should be outlined. These plans should include potential problems and their solutions. Possible solutions to some

problems frequently encountered during offshore surveys are listed in Table 1. Development of contingency plans can be greatly assisted by reviewing cruise summary reports and consulting with chief scientists of previous cruises.

The captain of the survey vessel should be provided with a copy of the survey plan to ensure that it is consistent with the equipment and capabilities of the vessel. Modifications to the ship or cruise plan may be required.

To ensure that all required sampling equipment and supplies are available at the time of sampling, an equipment checklist should be constructed. Spare parts and backup supplies should be included in the inventory.

TABLE 1. EXAMPLES OF PROBLEMS FREQUENTLY ENCOUNTERED DURING OFFSHORE SURVEYS AND RECOMMENDED SOLUTIONS TO EACH PROBLEM

Problem	Recommended Solution(s)
Sampling equipment fails or is lost overboard	- Have necessary tools onboard to make repairs - Maintain spare parts inventory for major equipment - Have back-up equipment onboard - Have SCUBA equipment and divers onboard for retrieval - Know where nearest tools, back-up equipment, or divers are located on shore
Sampling efficiency reduced by poor weather	- Extend cruise length - Reschedule cruise
Sampling efficiency reduced because of seasickness	- Ensure scientific crew is large enough to compensate for reduced personnel - See solutions to second problem
Sampling delayed because vessel is inoperative	- Reschedule cruise - Extend cruise length once vessel is repaired - Charter an alternate vessel
A sample is partially lost or slightly contaminated	- Discard and take another sample
Bottom sampler will not penetrate to a sufficient depth after repeated casts	- Add weight to the sampler - Move a short distance before taking next sample
One or more water bottles fail to trip on a particular cast	- Discard all samples from that cast and take a new cast

SAMPLING PROCEDURES

HEALTH AND SAFETY

Guarding the health and safety of the sampling team not only is mandatory in itself, but also ensures that such concerns do not interfere with the collection of quality data. It is recommended that all members of the sampling team wear hard hats and Coast Guard-approved personal flotation devices when working on deck. Whenever water sampling, grab sampling, and trawling are being conducted, the danger exists that the block, winch, or hydrowire will fail or that the water sampler, grab, or trawl assembly (especially the otter boards) will swing out of control. Any one of these events could knock someone unconscious (or worse), and possibly overboard.

When sampling in areas that are contaminated by toxic materials, special equipment (e.g., detectors, respirators) may be required by federal or state regulations. The use of plastic safety glasses when working with sediments is a good practice. Sediment particles introduced to the eye can cause abrasion, infection, or chemical contamination.

Perhaps the most common ailment when sampling is "mal de mer" (i.e., seasickness). Although much humor has evolved at the expense of the seasick, sample quality can be seriously compromised by impaired deck hands. It is therefore recommended that members of the sampling team who are prone to seasickness take appropriate medication (e.g., dramamine, scopolamine) prior to the cruise.

STATION LOCATION

Accurate navigation is essential to ensuring that stations can be plotted and reoccupied with a high degree of certainty. Although a variety of navigation and/or position fixing systems are available currently, factors such as price, availability, and accuracy vary considerably among them. The station positioning system selected for a given survey should be able to meet all study design requirements for accuracy and should, at a minimum, provide a high degree of precision (i.e., repeatable measurements). Positioning systems that are precise but lack a high degree of accuracy may be used after actual station locations are determined by accurate, independent means (i.e., "ground-truthed"). For bottom-related samples, all positioning systems should be used in conjunction with a fathometer to ensure that sampling occurs at the proper water depth (allowing for tidal stage and any fathometer corrections). The protocols for station positioning presented in a later chapter of this notebook should be consulted for details related to locating stations.

SAMPLE HANDLING

After sample collection, proper sample handling ensures that changes in the constituents of interest are minimized and guards against errors when shipping and analyzing samples. All stages of sample handling should be documented adequately. Documentation helps ensure that all sample handling requirements are carried out and serves as proof that handling was conducted properly if questions arise later.

FIELD PROCEDURES

It is important throughout any sampling and analysis program to maintain integrity of the sample from the time of collection to the point of data reporting. Proper chain-of-custody procedures allow the possession and handling of samples to be traced from collection to final disposition. Documents needed to maintain proper chain-of-custody include:

- Field logbook -- All pertinent information on field activities and sampling efforts should be recorded in a bound logbook. The field supervisor should be responsible for ensuring that sufficient detail is recorded in the logbook. The logbook should enable someone else to completely reconstruct the field activity without relying on the memory of the field crew. All entires should be made in indelible ink, with each page signed and dated by the author, and a line drawn through the remainder of any page. All corrections should consist of permanent line-out deletions that are initialed. At a minimum, entries in a logbook should include:

  - Date and time of starting work
  - Names of field supervisor and team members
  - Purpose of proposed sampling effort
  - Description of sampling site, including information on any photographs that may be taken
  - Location of sampling site
  - Details of actual sampling effort, particularly deviations from standard operating procedures
  - Field observations
  - Field measurements made (e.g., pH, temperature, flow)

- Field laboratory analytical results
- Sample identification
- Type and number of sample bottles collected
- Sample handling, packaging, labeling, and shipping information (including destination).

Chain-of-custody procedures should be maintained with the field logbook. While being used in the field, the logbook should remain with the field team at all times. Upon completion of the sampling effort, the logbook should be kept in a secure area.

- Sample labels -- Sample labels must be waterproof and must be securely fastened to the outside and/or placed inside each sample container (depending on the kind of sample) to prevent misidentification of samples. Labels must contain at least the sample number, preservation technique, date and time of collection, location of collection, and signature of the collector. Labels should be marked with indelible ink. Abbreviated labels may also be placed on the cap of each jar to facilitate sample identification.

- Chain-of-custody records -- A chain-of-custody record (Figure 1) must accompany every sample. Each person who has custody of the sample must sign the form and ensure that the samples are not left unattended unless secured properly.

- Custody seals -- Custody seals (Figure 2) are used to detect unauthorized tampering with the samples. Sampling personnel should attach seals to all shipping containers sent to the laboratory by common carrier. Gummed paper seals or custody tape should be used so that the seal must be broken when the container holding the samples is opened.

For further information regarding proper chain-of-custody procedures, consult the policies and procedures manual for the National Enforcement Investigations Center (NEIC; U.S. EPA 1978).

SAMPLE SHIPMENT

All preseved samples should be shipped immediately after completion of sampling. This minimizes the number of people handling samples, and protects sample quality and security. Guidance for shipping hazardous materials can be found in U.S. Department of Transportation (1984). As samples are prepared for shipping, the following should be kept in mind:

EPA

United States Environmental Protection Agency

Region 10
1200 Sixth Avenue
Seattle WA 98101

CHAIN OF CUSTODY RECORD

PROJECT				SAMPLERS: (Signature)							
LAB #	STATION	DATE	TIME	SAMPLE TYPE						NUMBER OF CONTAINERS	REMARKS
				WATER	SEDIMENT	TISSUE	AIR	OIL	OTHER		

RELINQUISHED BY: (Signature)	RECEIVED BY: (Signature)	DATE/TIME
RELINQUISHED BY: (Signature)	RECEIVED BY: (Signature)	DATE/TIME
RELINQUISHED BY: (Signature)	RECEIVED BY: (Signature)	DATE/TIME
RELINQUISHED BY: (Signature)	REC'V'D BY MOBILE LAB FOR FIELD ANAL.: (Signature)	DATE/TIME
DISPATCHED BY: (Signature) DATE/TIME	RECEIVED FOR LAB BY: (Signature)	DATE/TIME
METHOD OF SHIPMENT:		

Distribution Original – Accompany Shipment
One Copy – Survey Coordinator Field Files

Figure 1. An example of a chain-of-custody record.

U.S. ENVIRONMENTAL PROTECTION AGENCY
CLP Sample Management Office
P.O. Box 818 - Alexandria, Virginia 22313
Phone: 703/557-2490 - FTS/557-2490

SAS Number

SPECIAL ANALYTICAL SERVICE

PACKING LIST

Sampling Office:	Sampling Date(s):	Ship To:	For Lab Use Only
Sampling Contact: (name)	Date Shipped:		Date Samples Rec'd:
(phone)	Site Name/Code:	Attn:	Received By:

	Sample Numbers	Sample Description i.e., Analysis, Matrix, Concentration	Sample Condition on Receipt at Lab
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
16.			
17.			
18.			
19.			
20.			

For Lab Use Only

EPA CUSTODY SEAL

ENVIRONMENTAL PROTECTION AGENCY	SAMPLE NO.	SEAL BROKEN BY	DATE	EPA Form 7500-2 (10-71)
	SIGNATURE			
	PRINT NAME AND TITLE (Inspector, Analyst or Technician)			

Figure 2. Examples of a sample analysis request form (above) and a custody seal (below).

- Shipping containers should be in good shape and capable of withstanding rough treatment during shipping.

- Samples should be packed tightly:

  - Dividers must separate all glass containers

  - Empty space within shipping boxes should be filled so that jars are held securely.

- All containers must be leak-proof. If a container is not leak-proof by design, the interior should be lined with two heavy-duty plastic bags and the tops of bags should be tied once samples are inside. Adequate absorbent material should be placed in the container in a quantity sufficient to absorb all of the liquid.

- All samples should be accompanied by a sample analysis request. Variables to be analyzed by the laboratory, and total number and kind of samples shipped for analysis should be listed on the request sheet. An example sample analysis request form is illustrated in Figure 2. The laboratory should acknowledge receipt of shipment by signing and dating the form, and returning a copy to the designated QA coordinator.

- A chain-of-custody record for each shipping container should be filled out completely and signed.

- The original chain-of-custody record and analysis request should be protected from damage and placed inside the shipping box. A copy of each should be retained by the shipping party.

- The custody seal should be attached so that the shipping box cannot be opened without breaking the seal.

- For shipping containers carrying liquid samples:

  - A "This End Up" label should be attached to each side to ensure that jars are transported in an upright position.

  - A "Fragile-Glass" label should be attached to the top of box to minimize agitation of samples.

- Shipping containers should be sent by a carrier that will provide a delivery receipt. This will confirm that the contract laboratory received the samples and serve as a backup to the chain-of-custody record.

- All shipping charges should be prepaid by the sender to avoid confusion and possible rejection of package by the contract laboratory.

LABORATORY PROCEDURES

At the laboratory, one person should be designated custodian of all incoming samples. An alternate should also be designated to serve in the custodian's absence. The custodian should oversee the following activities:

- Reception of samples
- Maintenance of chain-of-custody records
- Maintenance of sample tracking logs
- Distribution of samples for laboratory analyses
- Sending of samples to outside laboratories
- Supervision of labeling, log keeping, data reduction, and data transcription
- Storage and security of all samples, data, and documents.

Upon reception of samples, a designated laboratory person should fill out the chain-of-custody record, indicating time and date of reception, number of samples, and condition of samples. All irregularities indicating that sample security or quality may have been jeopardized (e.g., evidence of tampering, loose lids, cracked jars) should be noted on the sample analysis request form and returned to the client-designated QA coordinator. In addition, a designated laboratory person should initiate and maintain the sample tracking log that will follow each sample through all stages of laboratory processing and analysis.

Minimum information required in a sample tracking log includes the following:

- Sample identification number
- Location and condition of storage
- Date and time of each removal of and return to storage
- Signature of person removing and returning the sample
- Reason for removal from storage
- Final disposition of sample.

All logbooks, labels, data sheets, tracking logs, and custody records should have proper identification numbers and be accurately filled out. All information should be written in ink. Corrections should be made by drawing a line through the error and entering the correct information. Corrections should be initialed and dated. Accuracy of all data reductions and transcriptions should be verified at least twice. All samples and documents should be properly stored within the laboratory until the client authorizes their removal. Security and confidentiality of all stored material should be maintained at all times. Before releasing analytical results, all information on sample tags, data sheets, tracking logs, and custody records should be cross-checked to ensure that data pertaining to each sample are consistent throughout the record.

Originals of the following documents should be sent to the client:

- Chain-of-custody records
- Sample tracking logs
- Data report sheets
- Quality control records.

Copies of all forms should be retained by the laboratory in case originals are lost in transit.

SHIPBOARD LABORATORY ANALYSES

Depending upon the size and capabilities of the survey vessel, many environmental variables can be analyzed on board. In general, the laboratory procedures described for the protocols in this document are applicable to both shipboard and land-based laboratories. This consistency is important to ensuring that analytical results will be comparable regardless of which kind of laboratory generates them.

Although most laboratory procedures are similar between shipboard and land-based laboratories, a number of additional factors must be considered when analyzing samples at sea. These factors relate primarily to the remoteness of the shipboard laboratory from land-based support and the movement and limited space of the survey vessel. The major considerations are as follows:

- The design of the laboratory should be efficient, with convenient equipment locations and adequate storage space.
- The vessel should be equipped with an uninterruptible power supply that is adequate for operation of all scientific instruments.

- The laboratory should be well ventilated to remove any toxic vapors created by chemicals.

- The temperature of the laboratory should be controlled, especially if variations in ambient temperature can influence particular analyses.

- Adequate lighting is necessary, especially for analyses requiring color discrimination (e.g., titration endpoints).
- The laboratory should have adequate water purification apparatus or be capable of storing water purified on shore.

- For storing many kinds of samples, adequate refrigeration and freezing capabilities are desirable.

- The laboratory should never be used as a general passageway or lounge.

- The laboratory should be off limits to unauthorized personnel.

- Adequate safety and first aid equipment should be on board, preferably including an overhead quick-pull safety shower.

- Extreme care must be taken when handling samples (for quality purposes) and hazardous reagents (for safety purposes), as vessel movement can sometimes be unpredictable.

- Backup supplies and instruments should be on board so that sampling can continue if a piece of equipment is broken or will not operate properly. A continuously updated inventory tracking system is useful for maintaining backup equipment.

- All equipment should be properly secured to compensate for predictable and unpredictable vessel movements. Specially designed racks are useful for this purpose.

- Instruments should be checked and calibrated before sailing, so that problems requiring land-based assistance can be solved quickly.

- Whenever possible, plastic containers should be used instead of glass because plastic is less susceptible to breakage.

- Instruments having digital displays are preferred over those using needles.

- Pre-printed data sheets should be used to ensure that all required information is recorded.

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U.S. Environmental Protection Agency. 1978 (revised 1983). NEIC policies and procedures. EPA-330/9-78-001-R. National Enforcement Investigations Center, Denver, CO.

STATION POSITIONING

FINAL REPORT
TC-3090-05

Puget Sound Estuary Program

RECOMMENDED PROTOCOLS FOR STATION POSITIONING IN PUGET SOUND

Prepared by:
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Prepared for:
RESOURCE PLANNING ASSOCIATES

for:
PUGET SOUND DREDGED DISPOSAL ANALYSIS (PSDDA)

Monitored by:
U.S. Army Corps of Engineers
Seattle District
Seattle, WA

August, 1986

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INTRODUCTION

This chapter presents recommended procedures for station positioning during environmental sampling in Puget Sound. Specifically, the objectives of this chapter are to:

- Recommend methods for selecting station positioning methods
- Recommend positioning accuracies and associated methods for specific kinds of sampling.

The recommendations in this chapter are based on the results of a workshop and written reviews by representatives of most agencies, institutions, and private firms that fund or conduct environmental research in Puget Sound (Table 1). At the workshop, it was agreed that no single positioning method would be adequate for all sampling scenarios. Instead, the group recommended development of procedures to select appropriate positioning methods for specific sampling objectives and to ensure consistent implementation of those methods. A standardized selection procedure was recommended that:

- Accommodates different sampling objectives and site-specific conditions
- Does not limit the positioning methods that can provide the accuracy needed or specified
- Allows investigators to use familiar or available systems
- Allows the funding agencies to specify levels of accuracy appropriate for sampling objectives or database entry
- Incorporates positioning accuracy as a component of sampling study design regardless of scientific approach.

The description of the selection procedure begins with a review of interrelationships between sampling program design and positioning requirements. Guidance for defining project-specific requirements is provided. The procedures and equipment that can provide the required accuracies are identified and an evaluation procedure for comparing candidate positioning methods is presented. Requirements to standardize the implementation of positioning methods and reporting of station locations are also presented. Finally, accuracy levels and corresponding positioning methods are recommended for three categories of sampling activities (areal samples, point samples, and special studies).

TABLE 1. CONTRIBUTORS TO THE STATION POSITIONING PROTOCOLS

Name	Organization
John Armstrong[a]	U.S. EPA
Gary Bigham[a,b]	Tetra Tech, Inc.
John Dermody[a]	Raven Systems
Ben Huntley[a]	Sea-Lease, Inc.
David Jamison[a]	WDNR
Karl Kassebaum	U.S. EPA
David Kendall[a]	U.S. COE
Gary Mauseth[a]	Nortec
Gary Minton[a]	Resource Planning Assoc.
Bill Muellenhoff[a]	Tetra Tech, Inc.
Bob Parker	U.S. COE
Tony Petrillo[a]	Nortec
Keith Phillips[a]	U.S. COE
Anthony Roth[a]	Cooper Consultants
Debra Simecek-Beatty[a]	NOAA
Jeff Stern[a,b]	Tetra Tech, Inc.
Steve Tilley[a]	WDNR
Bruce Titus[a]	URS Engineers
Frank Urabeck	U.S. COE
Pete Wilkinson[a]	Battelle Northwest

[a] Attended the workshop held on January 27, 1986.

[b] Workshop moderators.

POSITIONING METHOD SELECTION PROCEDURE

A procedure for identifying the positioning accuracy appropriate for a specific sampling objective and the corresponding positioning methods is presented in this section. The procedure involves identifying specific factors of the sampling program that affect the accuracy and feasibility of positioning methods. Next, the individual stations of the sampling program are used to determine the specific positioning requirements. Available systems are then evaluated based on positioning requirements and site-specific constraints to select an appropriate positioning method. These steps are followed by approval and procurement of the selected system, assembly and installation of shore stations (if needed), and equipment calibration and field-testing. The procedure is presented graphically in Figure 1.

DEVELOPMENT OF SAMPLING PROGRAM

Study design and location are important factors in the evaluation of positioning methods for environmental sampling vessels. Study design affects required positioning accuracy and location affects feasibility and accuracy of some positioning methods. Specific design and location factors include physical conditions and topography of the study site, proposed equipment and analyses, minimum station separation, station reoccupation, and program-imposed constraints. All of these factors need to be considered prior to sampling to ensure that positioning methods do not compromise quality or interpretation of the data. The design factor most sensitive to station location (e.g., requiring the highest positioning accuracy) will initially define the positioning requirements. This initial estimate should be re-evaluated during the study design to determine whether positioning limitations will require changes in the sampling program.

The type of positioning accuracy that is needed to meet the specific sampling objectives of each study should be identified during the evaluation of effects of design and location factors on positioning requirements. Absolute or predictable accuracy refers to a method's ability to correctly define a position by latitude and longitude (Bowditch 1984). Repeatable or relative accuracy measures a method's ability to return the user to the same position time after time. The difference between these two accuracies can be significant. Some of the study design or location factors may affect one type of accuracy but not the other. Examples are presented under each subheading below. It is important to identify which accuracy is of concern for positioning during a specific sampling program before finishing the following evaluation of effects on study design.

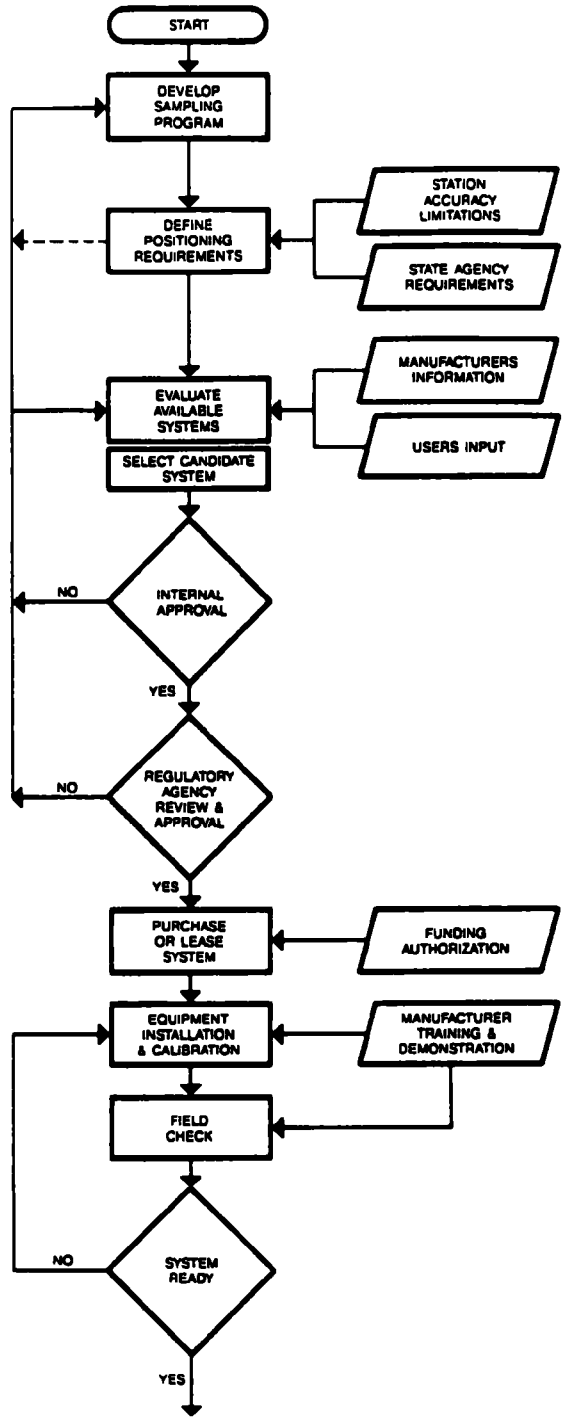


Figure 1. Positioning method selection procedure.

Physical Conditions at the Study Site

The ability of a positioning method to achieve its highest projected accuracy depends, in part, on site-specific conditions. The accuracy of a position fix from any two points increases as the angle between the lines-of-position (LOP) on which the vessel must lie approaches 90 degrees. The area of probable location that can be resolved by a navigation method (the radial error) varies among locations because of changes in the relative location of the fix points and the vessel. The number of objects or targets with known locations that can be used for a position fix depends on the methods used and, in some cases, on the surrounding terrain. An acceptable fix target for one method may not be an acceptable target for another method. A preferred method may not be usable or sufficiently accurate at all locations. For example, Loran-C cannot be used in some parts of Puget Sound (see Figure A-1, Appendix A) and the accuracy of visual sighting methods decreases with distance from shore. Thus, the location (or the combination of locations) of the study is a principal determinant in the usefulness of a specified positioning method. Effects of location on the accuracy and applicability of various positioning methods are described in Appendix A for optical, radar range, and short-range and long-range electronic positioning methods, which are commonly used in Puget Sound.

Weather, currents, and other physical factors may also reduce the achievable accuracy of a positioning method. For example, the relative drift of the sampling equipment away from the boat under strong currents or winds can increase with depth. Resulting positioning errors in sample location (as opposed to boat location) may exceed acceptable limits for the study if effects of site location on positioning accuracy are not considered during design of the sampling program.

Equipment and Analyses

The vessel available for the sampling program may affect the study design by limiting the types of positioning equipment that can be deployed or set up on board. If a positioning system already exists on the vessel, it should be evaluated to determine whether its accuracy is adequate for the sampling program.

Different levels of accuracy are required for different kinds of sampling. Water column sampling generally does not require a precisely known station location because the water column is relatively homogeneous compared with sediments. Trawling transects do not require high positioning accuracies because the sampled area is large and because the precise location of the net at any specified moment is uncertain. Accuracy is much more important for sampling conducted with equipment that penetrates or rests on the bottom (e.g., cores, grabs). Heavier equipment will usually reduce wire angles (see discussion under "Define Positioning Requirements," below) and the area in which the sampler was probably located. For equipment left at one location for a period of time (e.g., current meters, sediment traps),

repeatable or relative accuracy to return to the same position is important for retrieval. Absolute or exact geodetic positions may be less critical. Sampling of point sources generally requires both high absolute accuracy for exact location of sources and high relative accuracy for proper definition of the spatial distribution of sediment pollutant concentrations.

The chemical or statistical analyses to which the collected samples are subjected should also be considered in determining the required navigational accuracy. If a gradient of environmental effects is suspected, but the analytical technique cannot measure small differences in the value of a specified variable, sampling stations can be located farther apart and a relatively less accurate positioning method can be used. However, within-station variability may be more difficult to discern using a less accurate positioning method. For variables with a patchy distribution, the patch size could be smaller than the area defined by the repeatable accuracy of the positioning method, resulting in replicates sampled across community or physical boundaries. These conditions may not be noticed in the field and could prevent correct interpretation of the data. Statistical comparisons with replicate samples (e.g., "synoptic" data, field replicates, time-series samples) from heterogeneous stations will deserve special attention. The effects of navigational positioning accuracy and the associated probable sampling area (area from which samples could have been collected) on statistical comparisons of data should be considered in the study design.

Station Separation

When sampling stations occur in a grid, along a gradient, or in highly heterogeneous areas, it is especially important to maintain the statistical separation between them. Minimum separation should be defined by the diameter of the probable sampling area. Spatial resolution of station locations (i.e., probable sampling area) is limited by positioning method accuracy, depth, and wire angle. The proposed positioning method can be evaluated to determine whether statistical separation for the proposed station configuration will be achieved. Probable sampling area is determined by calculating the positioning error at a 95 percent probability level and adding the effect of wire angle. Procedures for determining the spatial resolution of a single station and adequate station separation are presented later in this section under "Define Positioning Requirements."

Reoccupation

Station reoccupation (e.g., for replicates or time series samples) requires accurate positioning techniques to minimize the area sampled. The variance (s^2) of the measured variables (for a specified number of replicates) usually increases with the area sampled (Holme and McIntyre 1984). The increase in variance can mask trends or gradients that may actually exist. With less accurate positioning methods, more replicates are needed to properly characterize the region and replicate samples are more likely to have been collected from different patches within the probable

area sampled. Because repeatable accuracy is typically one order of magnitude greater than absolute accuracy for a specified navigation method, repeatable accuracy should be used to determine the probable area sampled at a reoccupied station. (If the exact geodetic position is important, a highly accurate system can be used for initial occupation and a less accurate method can be used for reoccupation. The repeatable accuracy of the less accurate method may approach the absolute accuracy of the more accurate method.)

Program-Imposed Constraints

Various program-specific constraints may affect the decision on positioning methods. Sampling time limitations may preclude more logistically demanding positioning methods. The proximity of the study and reference areas to one another may require the shore stations to be moved. Operator (e.g., investigator, vessel captain) experience with the proposed positioning system can affect performance. An experienced operator is more likely to arrange transponders (electronic systems) or landmarks (range or bearing methods) for complete coverage of the study area and is more likely to identify potential problem areas prior to the start of the survey. Contractual obligations may limit changes to the study design, establish maximum expenditures for station positioning, or require a minimum achievable accuracy for the survey. Unless the sampling design demands higher accuracy, funding agency specifications for positioning requirements should be observed.

DEFINITION OF POSITIONING REQUIREMENTS

Once a study design is drafted, project-specific positioning requirements can be established. Such requirements are influenced primarily by:

- Characteristics of the survey area (e.g., physical, geographic)
- Minimum required station separation
- Maximum acceptable area from which the replicate samples can be collected.

Physical and geographic characteristics of the survey area affect the applicability of various positioning methods and their achievable accuracies. For example, as noted earlier, signal interferences in some parts of Puget Sound may preclude the use of Loran-C as a positioning method. Accuracy of other methods may be insufficient because objects by which to establish the position fix (shore stations or targets) are inaccurately mapped or unavailable. Map accuracy and the ability to locate on a map the shore stations or objects from which fixes were taken are the largest potential sources of positioning error. No matter how accurate the positioning methods are, the locations of sampling stations will always be relative to the location of fix points on the map used. If the map is not accurate for locations of landmarks, the station positions determined from those

landmarks can only be relocated adequately with the same methods. The exact or geodetic location determined for those stations will not be correct and other parties that try to relocate the stations using different positioning methods may sample a different location. Consequently, the most accurate, up-to-date map of appropriate scale should be used. Recent aerial photographs that include the survey area can be used to verify map accuracy. The latest editions of maps from the U.S. Geological Survey and the National Ocean Survey are generally of sufficient detail and accuracy for positioning purposes. "Blue line" maps produced by the U.S. Army Corps of Engineers are made from aerial photographs and should be used if available for the study area because of their higher accuracy.

Each station location should be plotted and numbered on the map. Type of sampling, water depth, estimated time on station, and frequency of occupation should be noted. Biological trawl paths and navigational hazards should also be indicated. The availability of known targets for visual or range fixes within acceptable range should be determined for each station. Candidate locations for shore stations for each positioning method should be examined. The selected locations should provide coverage of the entire sampling area. Estimates of position errors should be based on anticipated LOP or angle errors expected at each station. Limiting factors within the survey area and at individual sites should be summarized, based on an inspection of each candidate shore station site. Line-of-site obstructions, traffic frequency, competing transmitters, air-water boundary irregularities, accessibility, and security should be evaluated. These evaluations are expected to provide a basis for judging the feasibility of a specified positioning method.

Each station location is actually an area from which a sample could have been collected. The size of this area is determined by the error inherent in the positioning method (i.e., radial error), the vessel offset (i.e., distance from the hydrowire to the location on the vessel from which the station position was determined), and the wire angle (Figure 2). If one assumes a zero wire angle on the sample gear, the positioning method error will define the area from which the sample could have been collected. Any slight wire angle will increase the size of the area as a function of depth. The probable sampling area can be calculated for each specified level of probability, depth, wire angle, and accuracy, as explained in Appendix B, "Position Error Analysis." Probable sampling area should be calculated for the closest spaced and deepest stations at the 95 percent probability level [the sum of Equation 3 ($p=0.095$) and Equation 7 (wire angle effects) in Appendix B]. This sample area can then be used to determine the minimum spacing between stations that will guarantee statistical separation of stations. For studies where station overlap is not a problem, the probable sampling area can be calculated at a lower probability (using Equation 2 [$p=0.68$] in place of Equation 3). For example, assuming station overlap is not a problem (using the sum of Equation 2 and Equation 7), a vessel using a positioning system with an absolute accuracy of ± 2 m (± 6.6 ft) in 20 m (66 ft) of water could collect a bottom sample from anywhere within

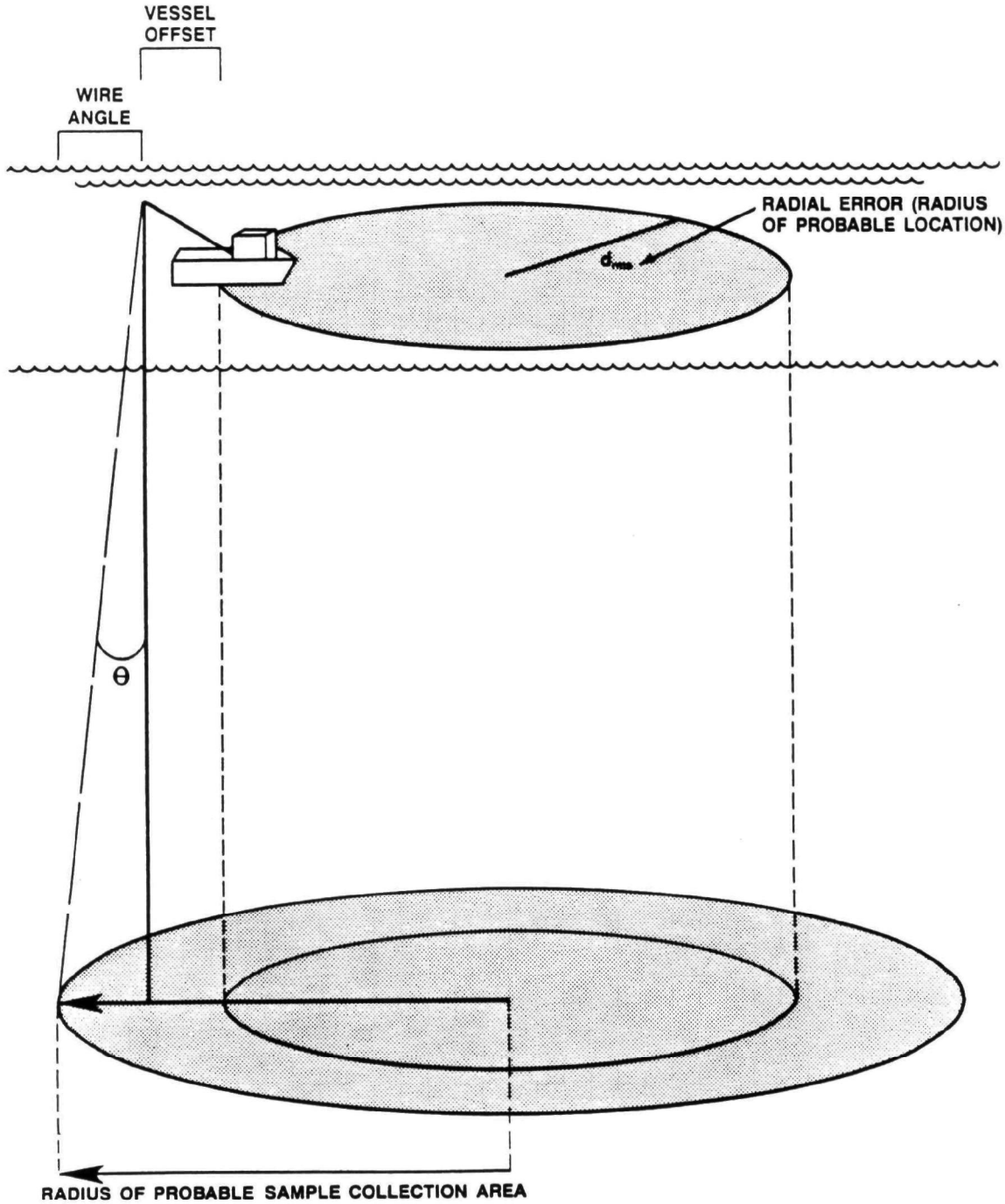


Figure 2. Components of error in probable area of sample collection.

a 66 m^2 (710 ft^2) probable sampling area. In 100 m (328 ft) of water, the probable sampling area would increase to 419 m^2 (4,508 ft^2).

Study objectives may require a small probable sampling area even if station separation is not a critical design feature. For example, a description of seasonal variation in benthic populations from a highly heterogeneous area of Puget Sound would require small probable sampling areas. The size of the probable sampling area should be determined by the scientific investigators on a case-by-case basis. Where station location and depth are fixed and the highest level of probability is assumed for the calculations, method accuracy is the only remaining variable.

Generally, it is sufficient to calculate probable sampling areas at three levels of accuracy: ±2, 20, and 100 m (±6.6, 66, and 328 ft) to determine the accuracy required for the survey. Both absolute and repeatable accuracies of positioning methods can be divided into these groupings, as will be demonstrated in the last section of this document, "Recommended Positioning Accuracies." Each positioning method will provide accuracies that could fall anywhere within a certain range depending on site-specific conditions. The ±2, 20, and 100 m (±6.6, 66, and 328 ft) accuracy levels are generally representative of the highest accuracies achievable under ideal conditions within the ranges of the various positioning methods. Candidate positioning methods can be evaluated by accuracy limitations to identify the most appropriate method. However, state agency or contractually required accuracies should never be exceeded. Having established accuracy requirements and survey area characteristics, the planner can then proceed with a detailed review of available systems.

REVIEW AND SELECTION OF POSITIONING METHOD

Positioning methods and associated equipment are described in detail in Appendix C. Additional information can be found in Ingham (1975), Umbach (1976), Maloney (1978), Bowditch (1984), and Tetra Tech (1986). Since any of these methods may be appropriate for a specific study, all should be considered initially. The investigator can determine if a particular method will meet calculated accuracy requirements at the survey location in question. Methods that do not meet accuracy requirements or are inappropriate can be eliminated from consideration. The remaining methods should be examined to identify the most appropriate method for the specific study. A detailed evaluation of various positioning methods is presented in Appendix D.

Each evaluation procedure must be carefully tailored to the user's needs and to the specific sampling or monitoring objectives. Factors such as accuracy, range, cost (procurement or rental), portability, flexibility (i.e., range of conditions under which the system can operate), security, user recommendations, training, and service availability are basic to such an evaluation, but are not all-inclusive. The screening of positioning methods in Appendix D should be supplemented by project-specific considerations. Other important factors to consider are visibility restrictions

if night or foul weather work is expected, existing equipment on the vessel to be used, and the rental availability. If purchase of a system is warranted, additional factors include compatibility with existing equipment, ability to accommodate future system expansion, and availability of ancillary items (e.g., data logger, plotter, tracking monitor, or computer). Potential use of the system for other types of projects may also be relevant. However, with the expected rapid progress in satellite navigation systems, it may be desirable to procure a less costly, more labor-intensive system now and wait 2-5 yr for a satellite system. At a minimum, the positioning method adopted should provide the accuracy to meet funding agency requirements and to maintain station separation.

IMPLEMENTATION OF POSITIONING METHOD

Once a positioning method that is adequate for the specific sampling objective has been selected, the proper setup, calibration, and operational procedures must be followed to achieve projected accuracies. If the appropriate equipment is already on board the vessel or the positioning task is hired out, the responsible party of the cruise should be sure that at least one member of the field crew is familiar with the positioning method. If the scientific team is supplying the equipment, appropriate training or experienced personnel should be provided to ensure proper equipment operation and documentation of positioning data. A backup method should be available on short notice to avoid loss of ship time if the primary method fails. To ensure that station locations are accurately occupied regardless of method and that adequate documentation is available for other parties, record-keeping requirements should be established, as described in the following section.

RECORDKEEPING REQUIREMENTS

FIELD RECORDS

Adequate information to ensure consistent positioning and to allow reoccupation of stations for replicate sample collection or time-series monitoring should be kept in a field logbook. More detailed descriptions facilitate problem-solving and return to the station by the original crew as well as other parties. Required entries into the logbook for initial survey description, day log entries, and station log entries are addressed below.

Initial Survey Description

The positioning method and equipment used should be detailed in the field logbook. All changes or modifications to standard methods and equipment should be noted . Names of persons who set up and operate the equipment should be listed in case questions arise. Locations of on-board equipment and the reference point on the vessel where the position is actually estimated (e.g., antennae, sighting position) should be noted. Distance from the vessel reference point to the hydrowire should be estimated to quantify the vessel offset component of positioning error. The type of map that was used for positioning and its identification number or scale should also be recorded.

Shore station locations should be provided in detail sufficient to allow another party to occupy the same shore station. Shore stations should be located on a monumented point, a surveyed structure, or tied into the local horizontal control at two established benchmarks by surveys performed at the Third-Order Class I level or higher (Kissam 1981; Umbach 1976). Existing monumented points can be located from National Ocean Survey Triangulation Diagrams. Shore station descriptions should include the monumented point reference number, location relative to local surroundings, and access restrictions. A complete copy of the surveying notes should be included in the field logbook.

Day Log Entries

Day log entries should record all variations from methods, equipment, or personnel detailed in the initial survey description. All problems or irregularities should also be recorded along with the location, time, and operator. Any weather or physical conditions that might affect achievable accuracy should be noted. These records help to explain problems that may not be apparent in the field and can provide information with which

to recalculate erroneous station positions. Relocation of any shore station should be documented in the same detail as for the initial survey description.

All calibration data should be recorded in the day log. All manufacturer-recommended calibration procedures and frequencies should be observed. In addition:

- Electronic distance measuring systems (e.g., microwaves) should be calibrated over water between two monumented points at the start and end of a survey. The calibration should be performed at the same range expected for most stations. Calibration should be checked each day by recording the readout at the same location (single station systems) or while crossing the baseline between two shore stations (multiple station systems).

- Calibration of Variable Range Radar (VRR) and Loran-C should be checked each day by recording the readout at the same location. Two VRR ranges from the same two targets should be recorded.

- The optical components of shore-based stations should be calibrated at the start and end of a survey by recording the angle between the two baselines that run from the station location to two other monumented points. This orientation check should be repeated whenever the station is bumped or moved.

Station Log Entries

Each station location should be recorded in the coordinates or readings of the method used for positioning in sufficient detail to allow parties not present at the survey to reoccupy the station in the future. The positioning information should be recorded at the time of sample collection (vs. equipment deployment) and for every reoccupation of the station, even during consecutive replicate sampling. Trawl station positioning data should be recorded at the beginning and end of every trawl and at each significant change of direction. Supplemental positioning information that would define the station location or help subsequent relocation (e.g., anchored, tied to northwest corner of pier, buoy) should be recorded. If photographs are used for a posteriori plotting of stations, the roll and frame numbers should be recorded. Depth, time (tidal height), ship heading, and wire angle estimation should also be recorded for each occupation of a station.

Conditions at each station should be recorded and checked against the range of operating conditions known to produce optimal accuracy for the specified method. Examples of these specific requirements are presented below for common positioning methods:

- VRR - Three fixes from permanent objects should be recorded for each station. Fixes should all be from targets 0.16 to 6.4 km (0.1 to 4 mi) away and all from the same range scale on the VRR. Station positions should be supplemented with Loran-C coodinates, depth, and all visual line-of-site fixes available.

- Loran-C - The range of Loran-C coordinates for the survey area should be recorded. This helps the operator notice signal interference problems and signal jumps (10-skip) that may otherwise result in recording faulty coordinates for a station position. Both day and night coordinates should be recorded from one location inside the study area to check for a 0.1 usec day/night variation. Any fluctuations at the 0.1 usec level while on station should be recorded, including the reading that appeared on the display the majority of the time.

- Microwave systems - Crossing angles of LOPs should be between 35 and 150 degrees for all stations. Readout should be noted two to three times before being recorded to eliminate multipath errors.

REPORTING REQUIREMENTS

Sampling data reports supplied to other parties should include rationale for selecting the positioning method and recordkeeping standards observed during data collection. Any specific problems (e.g., wind, currents, waves, visibility, electronic interferences) that resulted in positioning problems and those stations affected should be identified. Estimates of the accuracy achieved during the survey should be included. This information will help the reader to determine the quality and consistency of the sampling.

Station locations should be reported in State Plane coordinates (specify north or south zone) or by latitude and longitude (to the nearest second). Coordinates need not be reported for each replicate collected; a single set of coordinates for the station is sufficient. Depth corrected to mean lower low water (MLLW) should be supplied for each station. Coordinates for the start and end of each trawled section should be provided.

RECOMMENDED POSITIONING ACCURACIES

Accuracy requirements for different types of environmental sampling requirements are examined in this section. Sampling that requires highly accurate positioning methods to help ensure quality and consistency in collected samples and in data interpretation and analysis is described. The corresponding acceptable positioning methods are identified. This process is presented for two specific sampling objectives identified by the Puget Sound Dredged Disposal Analysis (PSDDA).

CLASSIFICATION OF SAMPLING

The sampled medium and collection method can be used to classify sampling activities. The collected sample will be representative of either a point location or a larger area. Examples of point and areal samples are provided in Table 2. For purposes of identifying accuracy requirements for sampling activities, samples collected from a homogeneous medium (e.g., water column) are considered representative of a larger area and thus similar to an areal sample. A third classification of sampling activities which could include either point or areal samples is also considered in Table 2. This third group (referred to in this document as special studies) consists of studies usually used to identify induced effects on the environment. Characterization of natural variability will be important for special studies and point samples collected from heterogeneous environments. Accuracy of the positioning method (i.e., error associated with station location, or spatial resolution of the location from which the sample could have been collected) will affect the ability to identify the difference between natural variability and induced effects. Other sampling will not require this level of accuracy. Therefore, levels of positioning accuracy can be recommended for the three classifications of sampling that are identified from Table 2 (special studies, point sampling at the sediment boundary, and areal sampling including any samples from the water column).

RECOMMENDED ACCURACY

Accuracy levels of the methods detailed in Appendix C and evaluated in Appendix D can be categorized as ±2, 20, or 100 m (±6.6, 66, and 328 ft) for the reasons discussed earlier in this document. The highest accuracy level [±2 m (±6.6 ft)] is recommended for sampling performed under special studies in Table 2, point sampling studies in Table 2, and studies designed to build the developing database for the Puget Sound Estuary Program. The highly accurate station locations possible using ±2-m (±6.6-ft) accuracy will allow more closely spaced stations and better detection of trends or gradients that could otherwise be masked by variance in replicate samples collected using less accurate positioning methods. In addition, uniformity

TABLE 2. CLASSIFICATION OF COMMON TYPES OF SAMPLING

Point Sampling

Water Column[a]

- Current measurements
- Water samples
- Sediment traps

Sediment Boundary

- Sediment physical/chemical
- Bioassay sediments
- Interstitial waters
- Benthic infauna
- Bivalve coring

Areal Sampling

Water Column

- Plankton tows
- Pelagic fish trawls

Sediment Boundary

- Demersal fish trawls
- Crab pots or trawls
- Bivalve dredging

Special Studies[b]

Point source investigations
Time series sampling
Synoptic collections
Spatial gradients (including surface microlayer, dye studies)
Station grids
Dredging site sediment characterizations

[a] Homogeneous nature of water column makes all water column sampling equivalent to areal sampling.

[b] Studies in this classification may contain any of the sampling types listed above but are considered separately because of additional accuracy requirements.

of positioning accuracy across these studies is expected to improve comparability of data and is one of the factors that affects overall data quality.

However, if the location or variable sampled does not require the highest accuracy levels, a lower accuracy level [±20 m (±66 ft)] may be adequate. For example, results from sampling the relatively homogeneous benthos of deeper waters would probably not be adversely affected if the replicate grabs were collected from a larger area. If ±20-m (±66-ft) accuracy is sufficient to address the objective in question, and the funding agency cannot justify additional funds for the use of higher accuracy in order to conform to the database requirement, the lower accuracy may be appropriate. In many cases, point sampling studies could be adequately performed at the lower level of accuracy. Such exceptions should be evaluated on a case-by-case basis.

The ±20 m (±66 ft) accuracy level should be adequate to collect areal samples. The more homogenous nature of the medium and larger area sampled make higher accuracies unnecessary. The exception would be water samples collected near point sources where location is important in determining dilution characteristics. The lowest accuracy level [±100 m (±328 ft)] generally is not recommended.

RECOMMENDED POSITIONING METHODS

Positioning methods that can achieve the two recommended accuracy levels are presented in Table 3. Approximate ranges of both absolute and repeatable accuracies for some of the methods are compared in Figure 3. Methods with ±2 to 10 m (±6.6 to 33 ft) accuracy are acceptable for the higher level of accuracy. Those with ±20 to 40 m (±66 to 132 ft) accuracy are acceptable for the lower level of accuracy. In addition, some of the advantages and disadvantages for these positioning methods are presented in Table 3. Absent from Table 3 are some of the optical positioning methods discussed in Appendix C. While these methods do not offer the accuracy necessary for most sampling farther from shore, they can be highly accurate in areas within 0.5 km (1,640 ft) of shore. Along the waterfront of urban areas and in waterways where other methods may be unsuitable because of interference or minimum range requirements, optical positioning techniques may be preferable. Optical range finders and visual line-of-sites can provide accurate positioning if detailed notes are taken. Positions may also be determined by taking three photographs to allow for a posteriori triangulation from aerial maps or "blue lines." Any positioning by optical methods is improved if multiple methods (e.g., photographs) are employed.

The lead scientific investigator should decide whether the limiting factor in study design is absolute or repeatable accuracy, what effects the location will have on positioning capability, and determine which one of the candidate methods is appropriate for the specific sampling program. The following determinations of appropriate accuracy levels for specific

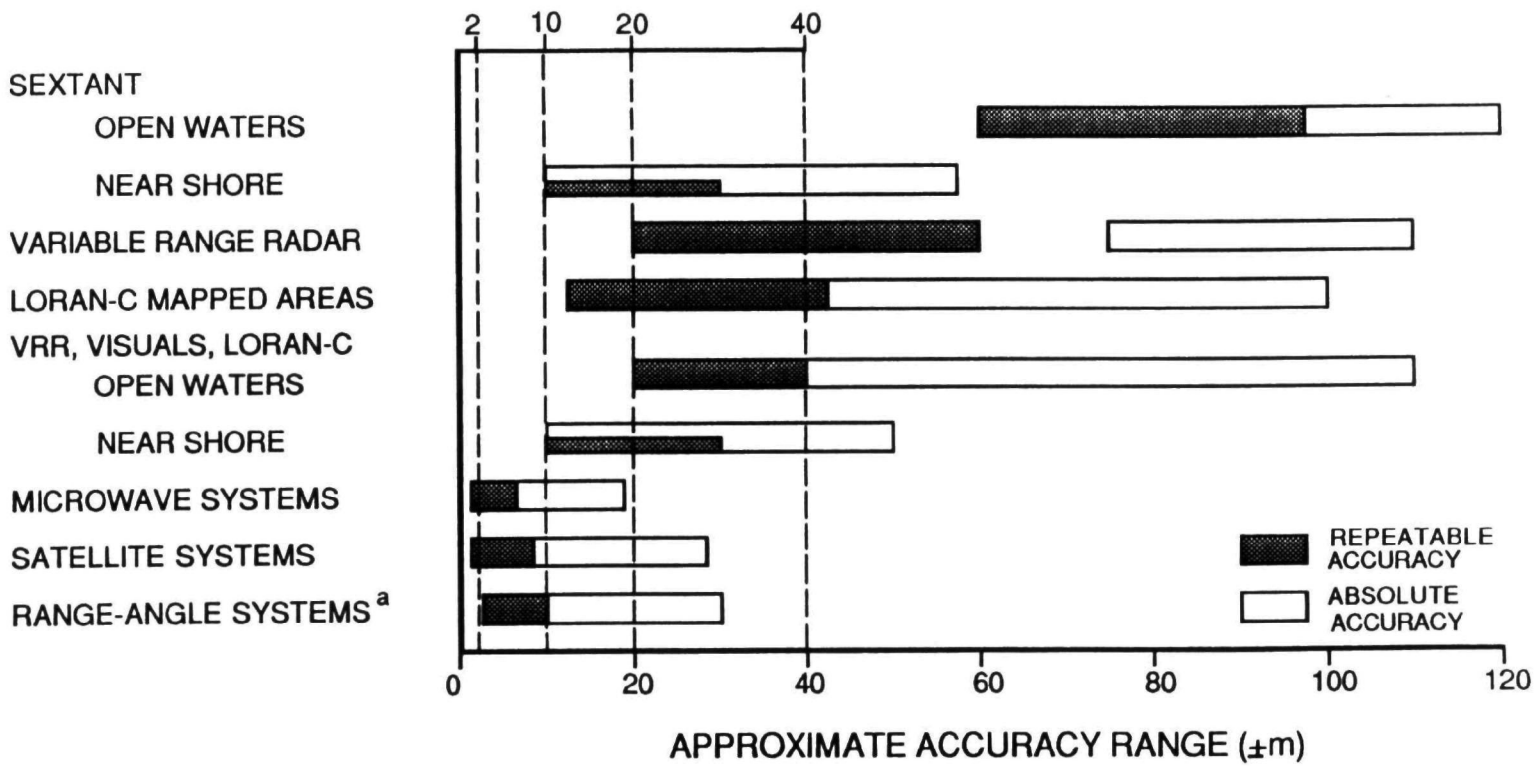
TABLE 3. SUMMARY OF ACCEPTABLE POSITIONING METHODS

Category	Representative Equipment	Achievable Accuracy	Maximum Range	Cost	Advantages	Disadvantages
Higher Accuracy Group						
Theodolite	Table C-2 Table C-3	10-30 sec ± 1 m and up	<5 km	$1,000-$4,000	Traditional method. Inexpensive. High accuracy. Successfully applied. Restricted areas.	Line-of-sight. Two manned shore stations. Simultaneous measurements. Limits on intersection angles. Area coverage; station movement.
EDMI	Table C-7	1.5-3.0 cm	3 km without multiple prisms	$3,500-$15,000	Extremely accurate. Usable for other surveying projects. Cost. Compact, portable, rugged.	Motion and directionality of reflectors. Line-of-sight. Visibility, unless microwave. Two shore stations. Ground wave reflection.
Total Stations	Table C-8	5-7 cm	<5 km	$8,000-$30,000	Single onshore station. Other uses. Minimum logistics.	Reflector movement and directionality. Prism costs. Line-of-sight. Optical or infrared range limitations.
Microwave Navigation Systems	Table C-9	± 1-3 m	25 km	$40,000-$90,000	No visiblity restrictions. Multiple users. Highly accurate. Radio line-of-sight.	Cost. Multiple onshore stations. Logistics. Security. Signal reflective nulls.
Range-Azimuth Systems	Table C-12	0.01° and 0.5 m	<5 km (optical) 30 km (elec)	$65,000-$100,000	High accuracy. Single station. Circular coverage.	Single user. Cost. Line-of-sight. Signal reflective nulls.
Satellite Systems	Table C-11	1-10 m	none	$150,000-$300,000 (initial units) under $10,000 later	High accuracy. Minimum logistics. Use in restricted/congested areas. Future cost. No shore stations.	Current coverage. Initial development cost.

TABLE 3. (Continued)

Category	Representative Equipment	Achievable Accuracy	Maximum Range	Cost	Advantages	Disadvantages
Lower Accuracy Group						
Sextant[a]	Table C-4	± 10 sec ± 2 m and up	<5 km	$1,000-$2,000	Rapid. Easy to implement. Common equipment. Low cost. No shore party. High accuracy closer to shore.	Simultaneous measurement of two angles. Target visibilities, location, maintenance. Line-of-sight. Best in calm conditions. Limits on acceptable angles.
Loran-C	Table C-11	± 15 m and up	none	$1,000-$4,000	No visibility or range restrictions. No additional personnel. Low cost, existing equipment.	Interference in some areas. Used only for repositioning except in limited areas, need to locate station initially with another system.
Variable Range Radar	Table C-5	± 0.5° ± 25 m	16-72 km	$4,000-$10,000	No visibility restrictions. No additional personnel. Low cost, existing equipment.	Line-of-sight. Relies on map accuracies of targets. Accuracy decreases with range scale.

[a] Accuracies greater than ±20 m are not common farther than 1 km from shore under normal operating conditions and is therefore considered in the lower accuracy group.



[a] INCLUDES TOTAL STATIONS AND RANGE-AZIMUTH SYSTEMS

Figure 3. Comparison of approximate accuracy ranges of some positioning methods.

sampling objectives are presented as examples of the process. Sampling studies conducted for PSDDA will consist of two basic objectives:

- To characterize the sediments to be dredged
- To monitor disposal-related impacts and trends at dredged material disposal sites.

Both objectives are included under special studies. The higher accuracy is recommended for sediment characterization because exact locations of the areas to be dredged must be defined for later removal of only the specified sediments. Dredged areas are usually close to shore. Most of the higher accuracy methods in Table 3 will meet these requirements close to land.

High repeatable accuracy is required for site monitoring to ensure that observed trends are temporal and not spatial. Most of these sites will be over 2 km from shore. Range restrictions may preclude some of the higher repeatable accuracy systems (see Appendix D; Figure D-1) at selected sites. Absolute position accuracy may not be important, although this determination will have to be made for each disposal site and for individual resources of concern. If absolute positioning is needed the higher accuracy methods are recommended.

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APPENDIX A

SITE-RELATED POSITIONING LIMITATIONS

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SITE-RELATED POSITIONING LIMITATIONS

Location has many effects on the accuracy and applicability of various positioning methods. Such effects are described below for optical, radar range, and short-range and long-range electronic positioning methods. These methods are described because they are commonly used in Puget Sound.

Optical positioning methods rely on the visual resolution of objects with a known position. Built structures provide more accurate fixes than land features because sharply defined objects provide better resolution. The ability to resolve an object decreases with distance. Within 5 km (3.1 mi) of the shoreline and in more developed areas, accuracy of optical methods can be comparable to that of electronic methods (Umbach 1976). However, optical methods are more dependent upon proper operation and target choice than are other methods. Urban embayments and waterways are therefore better suited for optical positioning than are regions in central Puget Sound or areas along less populated, featureless shorelines. The abundance of accurately located channel markers throughout Puget Sound provides good sightings in otherwise featureless areas. On featureless shorelines, a line tangent to the shoreline is used as a line-of-position, with considerable reduction in accuracy. Use of optical methods is restricted to daylight hours of good visibility.

Positioning by multiple ranges measured from a variable range radar system requires fixes on known positions, but eliminates visibility restrictions. However, because the radar signal is shadowed beyond the first object it strikes, the choice of targets can be limited. A second limitation is possible misidentification of reflection sources in developed areas. Positions based on misidentified reflection sources are inaccurately located, but can be reoccupied if the same perceived reflection source is subsequently used. A third target should be used to crosscheck position determined from two other targets. All three fixes should be on the same radar range scale.

Reflections depend on target position and alignment. The most accurate radar range fixes are based on reflections from objects 0.16-6.4 km (0.1-4 mi) distant (Crawford, P., personal communication). At stations farther from shore, adequate targets may not be available. Reflections closer than 0.16 km (0.1 mi) may be erroneous and should not be used. Sloped headlands and tidal flats are not usable as targets.

Short-range electronic positioning systems (e.g., microwave) involve at least two shore stations (transponders) and an on-board transmitter.

A set of stations is required for each study area. Successful reception of electromagnetic signals is the critical feature for effective operation of electronic positioning systems. Signals from the transponders or shore stations should be received at an angle of 30 to 150 degrees; 90 degrees is optimal. Signal reception is dependent upon electronic "line-of-sight" and may be blocked by tight quarters (e.g., waterways, rivers, and shorelines) and heavy vessel traffic. Such problems are alleviated by newer, more expensive systems that accommodate over a dozen transponders. With proper transponder locations, microwave systems can be used to position a vessel any distance from shore in Puget Sound except closer than 100 m (328 ft) to one of the transponder locations.

Accuracy of microwave systems depends upon placement of the remote transponders or shore stations. For example, remote stations not located on a monumented point will increase error of the vessel position fix. However, access restrictions, benchmark locations, and line-of-site considerations limit the available transponder locations and achievable angles. Permanent shore station locations are further limited by availability of power sources and site security.

Repeatable accuracy of microwave systems, while not affected by transponder location errors, is dependent upon the line-of-position angle. Certain combinations of transponder and vessel locations may result in signal cancellation (range holes) and failure to obtain a fix. Occurrence of range holes varies by location, is impossible to predict, and may force relocation of a shore station. In developed areas, reflections from metal objects or buildings may compound the problem or cause jumps in the received signal.

Long-range electronic positioning systems operate on permanent transmitting stations and user-carried receivers. The only receivable long-range system in Puget Sound is Loran-C. However, because land masses distort signal propagation, Loran-C charts are of unknown accuracy in inland waters such as Puget Sound. In addition, unidentified electronic sources interfere with Loran-C reception in some areas and prevents its use much of the time (Figure A-1). However, Loran-C is accurate for repositioning at locations where readings were recorded in Loran-C on original occupation. Some areas may exhibit a day/night signal variation of about 0.1 usec (Eaton C., personal communication) and should be checked when sampling at night.

Recent Loran-C maps based on comparison of Loran-C coordinates with those from other methods at the same stations support positioning to a resolution of 0.1 usec of the Loran-C signal [about 37 m (120 ft)] for limited areas in the vicinity of Elliott Bay (Sturgill, D., personal communication). Accurate maps for other areas are not available. Distortion can be defined for areas outside Elliott Bay only by taking readings at benchmarks around the shoreline. Accuracy is less reliable with distance from the benchmarks. Therefore, while Loran-C is usable around Elliott Bay for initial positioning, it can be used in other areas only as a repositioning tool and will require defining Loran-C station coordinates.

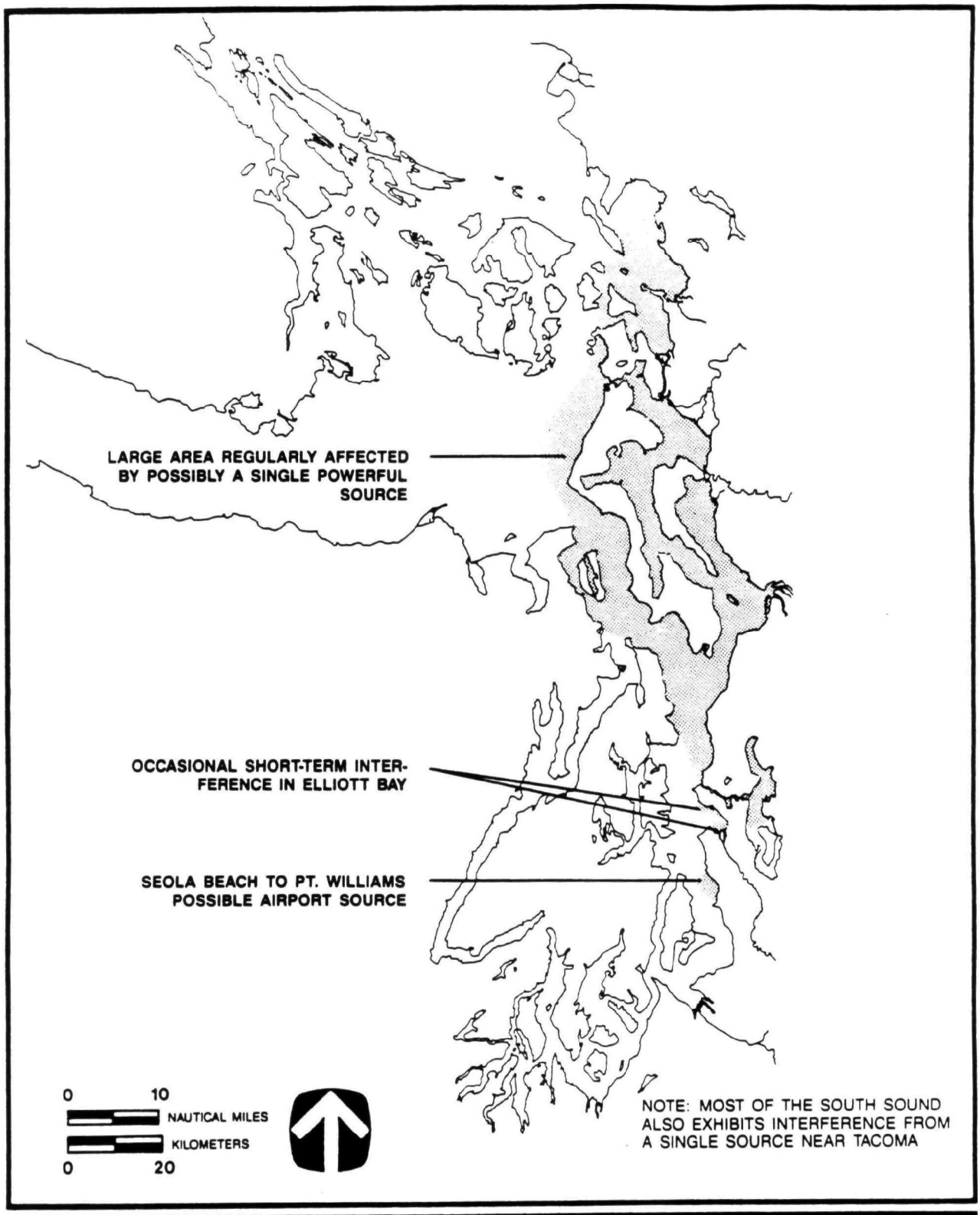

Figure A-1. Regions of LORAN-C signal interference in Puget Sound.

APPENDIX A

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APPENDIX B

POSITION ERROR ANALYSIS

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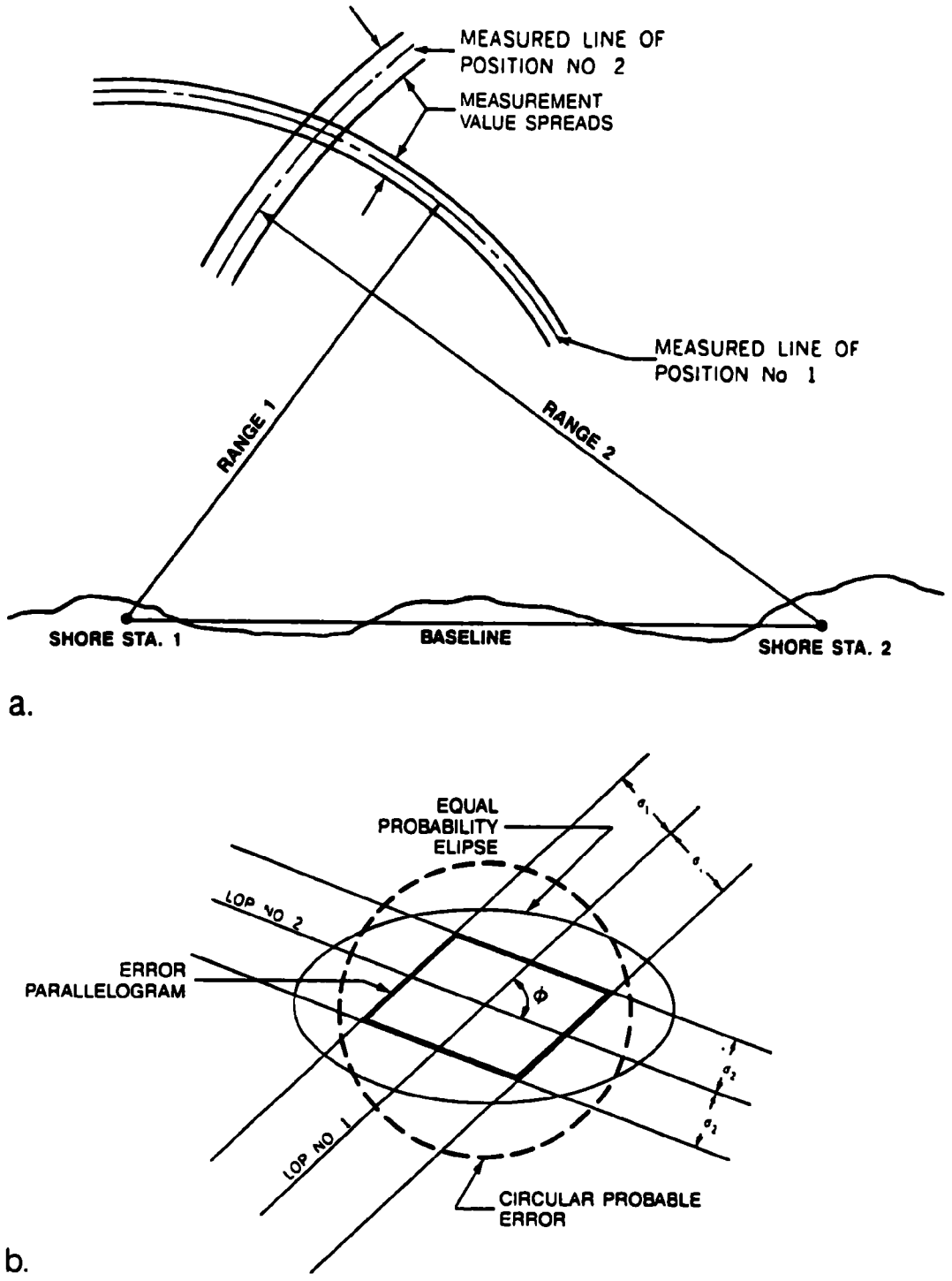
Errors have been traditionally classified in three categories: gross, systematic, and random (Mikhail and Gracie 1981). Gross errors are mistakes due to observer carelessness and must be detected and eliminated from positional measurements. Systematic errors occur due to a deterministic system, in this case the sextant, range finder, or electronic positioning equipment. Such errors can be expressed by functional relationships and can therefore be corrected for in the final measurement. Random errors reflect variations in measurements that remain after eliminating gross errors and allowing for systematic errors. As their name implies, such observational errors are random and have no known functional relationship. Random errors, the subject of the following discussion, are dealt with on a statistical basis using probability models.

All positional fixes are in error to some extent. This is true even if the transmitting stations have been very accurately located and bias or systematic errors in position coordinates have been eliminated. Random errors will still cause a variable displacement of position lines about their computed positions. Each method used to establish a position has inherent random errors that determine the overall accuracy of a fix. Absolute or predictable accuracy refers to a method's ability to correctly define a position by latitude and longitude (Bowditch 1984). Repeatable or relative accuracy measures a method's ability to return the user to the same position time after time. The difference between these two accuracies can be significant. Therefore, it is important to identify which accuracy is of concern for positioning during a specific sampling program before determining the error associated with that accuracy.

Positional accuracy is usually stated in terms of the probability of being within a certain distance of a desired geographical location. The line-of-position (LOP) can therefore be considered a strip whose width is a function of the LOP uncertainty, which can be described by its standard deviation (σ) (Figure B-1a). Because a fix involves at least two LOPs, each having an uncertainty, the LOP crossing boundary forms a parallelogram (Figure B-1b). The parallelogram area is dependent on the size of the standard deviations of the two LOPs and the angle at which they cross. The parallelogram area is given by:

$$A = (2\sigma_1)(2\sigma_2)\csc\phi = 4\sigma_1\sigma_2\csc\phi \qquad (1)$$

where σ_1 and σ_2 are the LOP measurement standard deviations. Because the cosecant function becomes large for angles less than 30 degrees and more



SOURCE: MODIFIED FROM BOWDITCH 1984

Figure B-1. Line of position measurements for two shore stations depicting LOP uncertainity (a), and associated error indicator (b).

than 150 degrees, so too does the size of the error parallelogram (Figure B-2). Thus, to reduce positional uncertainties, shore station locations should be located such that very large or very small cut angles do not occur within the survey area.

Probability theory indicates a 68.27 percent chance of being within $\pm 1.0\sigma$ of the mean of a set of normally distributed measurements. However, the probability that the measured position is within the parallelogram is less. For two sets of measurements, the joint or product probability is $(0.6827)^2 \times 100$ or 46.6 percent. A probable parallelogram is defined as one for which the probability of a measurement being within its boundary is 50 percent. This occurs at distances slightly larger than $\pm 1.0\sigma$ (i.e., $\pm 1.05\sigma$). Using $\pm 2.0\sigma$ results in a parallelogram within which it is 91 percent probable that the positional location occurs. The principal drawback is that the error parallelogram is not a curve of equal probability. An equal probability contour from a two-LOP fix is an ellipse centered on the LOP intersections. As with the parallelogram, the area and configuration of the error ellipse are dependent on the crossing angle and standard deviations in each set of measurements. The ellipse in which 50 percent of the expected measurements lie is referred to as the probable ellipse. Techniques for determining the orientation and the magnitudes of an error ellipse for various probability levels are presented by Mikhail and Gracie (1981).

It is more common to establish the probability that a position is located within a circle of a particular radius than to define an error ellipse. The resultant circular probable error (CPE or CEP) is the radius of a circle within which 50 percent of the fixes should be located. Bowditch (1984) provides two methods for determining the sizes of error circles at a desired probability level, given the standard deviation in each LOP measurement and the associated pattern crossing angle. The probability that the measured position is within a circle of a selected radius can also be determined.

When the standard deviations of two sets of measurements are equal and the cut angle is 90 degrees, the error figure becomes a circular normal distribution. Table B-1 can be used to determine the probability that location is within a circle of given radius from the measured position. At a radial distance (R) from the true position equal to 1σ (i.e., the ratio of $R/\sigma=1$), there is a 39.9 percent chance of being located within the circle (Table B-1). A 50 percent probability occurs for a circle with a 1.2σ radius ($R/\sigma=1.2$), and a 90 percent probability occurs for a circle with a 2.2σ radius. These probabilities change as the cut angle varies from 90 degrees. For example, if the cut angle is 40 degrees, a radius greater than 3.4σ is needed to achieve a 90 percent probability that the position lies within the circle boundary. Table B-1 can also be used to determine if positioning error is small enough to separate the error circles of two closely spaced positions. The probability of being located within a circle can be calculated (at any cut angle) for an error circle radius

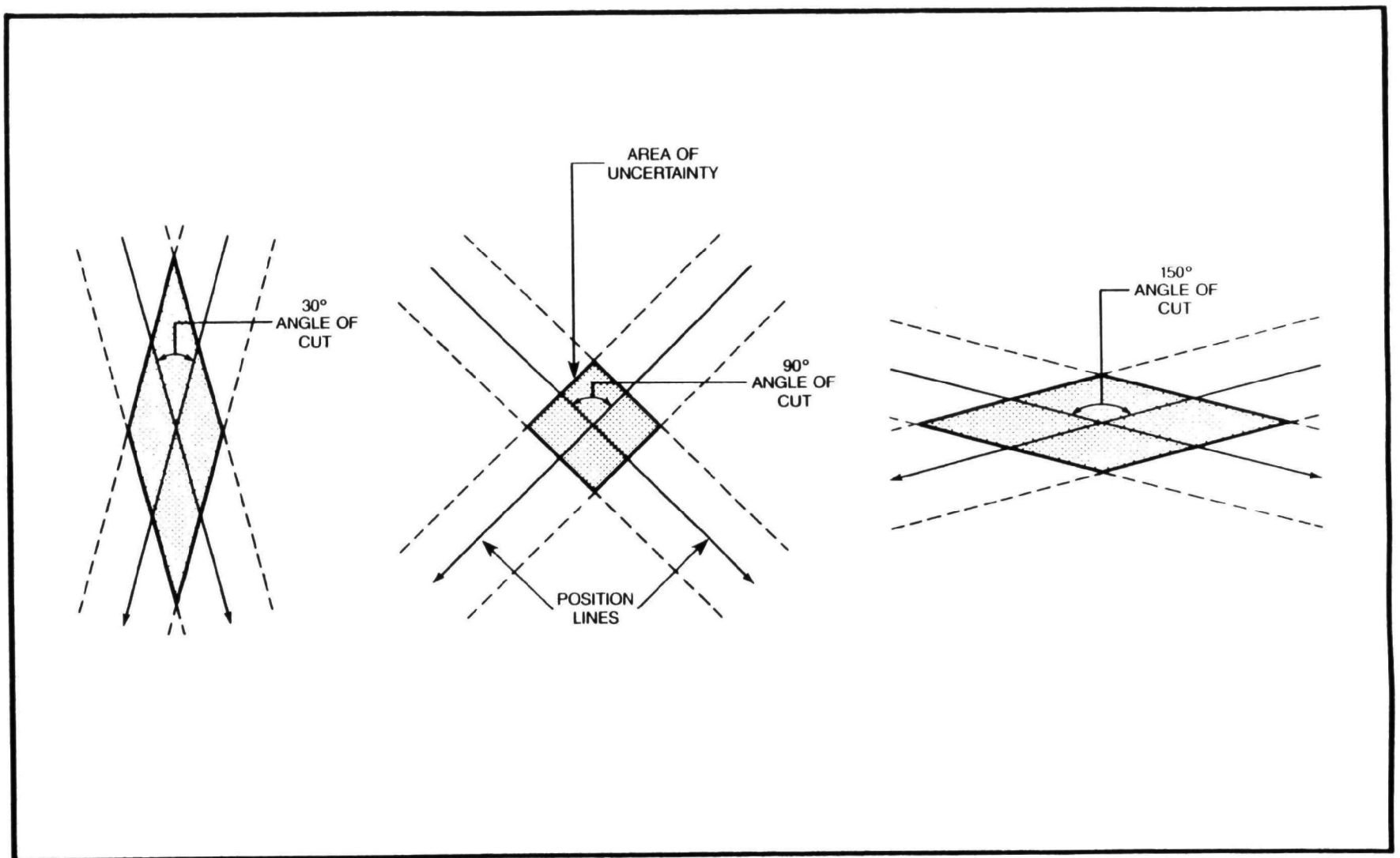


Figure B-2. Angle-of-cut effects on fix accuracy.

TABLE B-1. PROBABILITY VERSUS R/σ FOR ELLIPTICAL BIVARIATE DISTRIBUTIONS WITH TWO EQUAL STANDARD DEVIATIONS

R/σ \ φ	5°	10°	15°	20°	25°	30°	35°	40°	45°	50°	55°	60°	65°	70°	75°	80°	85°	90°
0.0																		.000
0.1																		.005
0.2	.001	.004	.006	.007	.009	.011	.011	.012	.014	.015	.016	.017	.018	.018	.019	.019	.019	.020
0.3	.005	.009	.012	.015	.019	.022	.025	.028	.031	.034	.036	.038	.040	.041	.042	.043	.044	.044
0.4	.008	.014	.021	.027	.033	.039	.045	.050	.055	.059	.063	.067	.070	.072	.074	.076	.077	.077
0.5	.009	.019	.029	.039	.048	.057	.066	.074	.083	.090	.096	.102	.106	.110	.114	.116	.117	.118
0.6	.014	.029	.043	.057	.070	.082	.095	.106	.117	.127	.135	.143	.150	.156	.159	.162	.164	.165
0.7	.020	.039	.057	.075	.093	.110	.126	.141	.155	.168	.179	.189	.198	.205	.211	.215	.217	.217
0.8	.025	.048	.072	.095	.117	.138	.158	.177	.195	.211	.226	.238	.249	.258	.265	.270	.273	.274
0.9	.031	.060	.088	.116	.143	.169	.196	.217	.238	.257	.275	.290	.303	.314	.322	.328	.332	.333
1.0	.035	.070	.104	.137	.169	.199	.228	.256	.281	.305	.326	.343	.358	.371	.381	.388	.392	.393
1.1	.040	.080	.120	.158	.196	.231	.265	.296	.325	.352	.366	.396	.414	.428	.440	.448	.452	.454
1.2	.046	.092	.136	.181	.224	.266	.302	.337	.370	.400	.426	.449	.469	.485	.497	.506	.512	.513
1.3	.053	.104	.155	.204	.251	.296	.339	.378	.414	.446	.475	.501	.522	.540	.553	.563	.569	.570
1.4	.058	.115	.171	.226	.278	.327	.374	.417	.456	.491	.523	.550	.573	.592	.606	.617	.623	.625
1.5	.063	.126	.188	.247	.304	.358	.408	.455	.497	.535	.568	.597	.621	.641	.656	.667	.673	.675
1.6	.069	.138	.204	.269	.330	.388	.442	.491	.536	.576	.611	.640	.666	.686	.702	.713	.720	.722
1.7	.074	.148	.220	.289	.355	.417	.471	.526	.573	.615	.650	.682	.707	.728	.744	.756	.762	.764
1.8	.079	.159	.236	.310	.380	.445	.505	.559	.608	.651	.688	.719	.745	.766	.782	.793	.800	.802
1.9	.084	.169	.251	.329	.403	.471	.536	.591	.641	.685	.722	.756	.780	.800	.816	.827	.833	.836
2.0	.089	.179	.266	.349	.426	.498	.563	.621	.672	.716	.753	.787	.810	.832	.846	.856	.862	.865
2.1	.093	.189	.280	.367	.448	.523	.589	.649	.700	.744	.780	.812	.837	.857	.871	.881	.887	.890
2.2	.099	.199	.295	.386	.470	.546	.615	.675	.727	.771	.808	.838	.861	.880	.894	.903	.909	.911
2.3	.104	.209	.309	.404	.490	.570	.639	.700	.752	.795	.831	.860	.883	.900	.913	.922	.927	.929
2.4	.109	.219	.324	.422	.512	.592	.663	.724	.775	.818	.852	.880	.901	.918	.930	.938	.942	.944
2.5	.115	.229	.338	.440	.532	.614	.686	.746	.797	.838	.871	.897	.918	.933	.943	.951	.955	.956
2.6	.119	.239	.352	.457	.551	.636	.708	.766	.816	.856	.888	.912	.931	.945	.954	.961	.964	.966
2.7	.124	.248	.366	.474	.570	.656	.728	.786	.835	.873	.904	.926	.943	.955	.964	.969	.973	.974
2.8	.129	.258	.379	.490	.589	.676	.746	.805	.851	.888	.916	.937	.953	.963	.971	.976	.979	.980
2.9	.134	.268	.393	.507	.607	.692	.763	.821	.867	.902	.928	.947	.961	.971	.978	.982	.984	.985
3.0	.139	.277	.406	.522	.624	.710	.781	.837	.880	.914	.938	.956	.968	.976	.982	.986	.988	.989
3.1	.144	.287	.419	.538	.641	.727	.797	.852	.893	.924	.947	.963	.974	.982	.986	.989	.991	.992
3.2	.149	.296	.432	.553	.657	.743	.812	.867	.905	.934	.955	.969	.979	.985	.990	.992	.993	.994
3.3	.154	.306	.445	.568	.673	.759	.826	.878	.916	.943	.962	.975	.983	.989	.992	.994	.995	.996
3.4	.159	.315	.458	.583	.688	.773	.840	.889	.925	.951	.968	.979	.986	.991	.994	.996	.997	.997
3.5	.164	.324	.470	.597	.703	.788	.852	.900	.934	.957	.973	.983	.989	.993	.995	.997	.998	.998
3.6	.169	.333	.482	.611	.718	.801	.864	.910	.942	.963	.977	.986	.991	.995	.997	.998	.999	.999
3.7	.174	.343	.494	.625	.731	.814	.875	.919	.949	.968	.981	.990	.993	.996	.998	.999	.999	.999
3.8	.179	.352	.506	.638	.744	.826	.886	.927	.955	.973	.984	.992	.995	.997	.998	.999	.999	1.000
3.9	.183	.360	.518	.651	.757	.838	.895	.935	.961	.977	.987	.993	.996	.998	.999	.999	1.000	1.000
4.0	.189	.370	.529	.664	.770	.848	.904	.942	.966	.980	.989	.994	.997	.998	.999	1.000	1.000	1.000
4.1	.193	.378	.541	.676	.781	.859	.913	.948	.970	.983	.991	.995	.998	.999	1.000	1.000	1.000	1.000
4.2	.198	.387	.552	.688	.793	.869	.920	.954	.974	.986	.993	.996	.998	.999	1.000	1.000	1.000	1.000
4.3	.203	.396	.564	.700	.804	.878	.927	.958	.978	.988	.994	.997	.999	1.000	1.000	1.000	1.000	1.000
4.4																		1.000

Reference: Bowditch 1984.

of one half the distance between the two positions to determine if position overlap may occur statistically.

Positional accuracy is often quoted using radial error, rms error, or d_{rms}, all of which have the same meaning. As with CEP, the error figure is a circle having a radius equal to:

$$d_{rms} = (\sigma_x^2 + \sigma_y^2)^{1/2} \tag{2}$$

where σ_x and σ_y are the 1σ error components along the major and minor axes of the probability ellipse (Figure B-3). This is often referred to as the $1d_{rms}$ or 1σ fix accuracy. Similarly, a 2σ fix is given by:

$$2d_{rms} = [(2\sigma_x^2) + (2\sigma_y^2)]^{1/2} \tag{3}$$

Given the LOP standard deviations σ_1 and σ_2, the standard deviations along the basic ellipse axes can be determined by:

$$\sigma_x^2 = (1/2 \sin^2\phi) \{\sigma_1^2 + \sigma_2^2 + [(\sigma_1^2 + \sigma_2^2)^2 - 4(\sin^2\phi)\, \sigma_1^2\sigma_2^2]^{1/2}\} \tag{4}$$

and

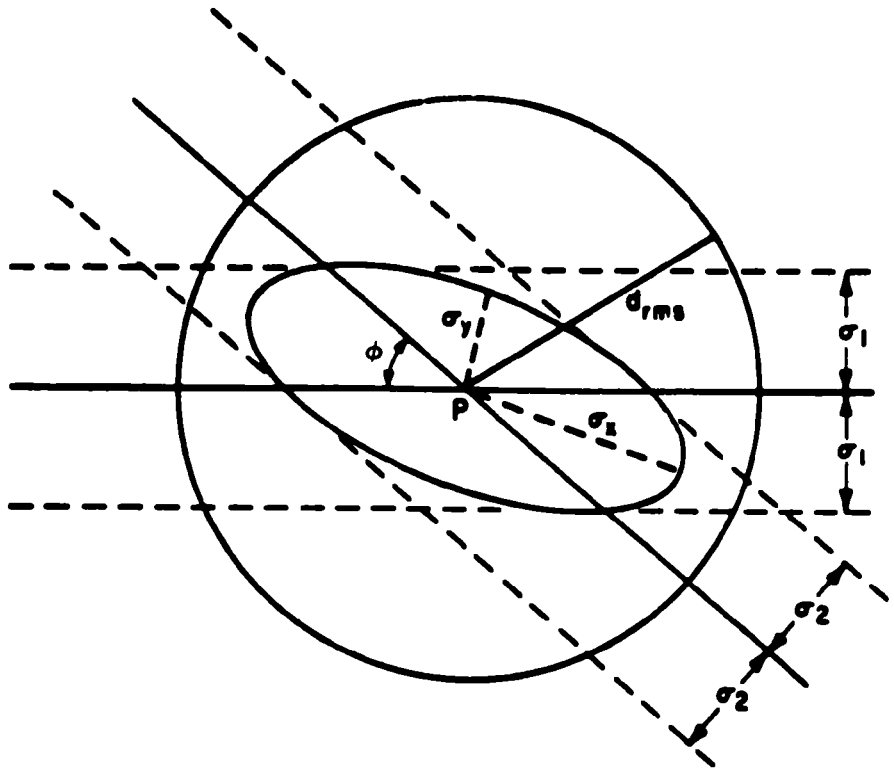
$$\sigma_y^2 = (1/2 \sin^2\phi) \{\sigma_1^2 + \sigma_2^2 + [(\sigma_1^2 + \sigma_2^2)^2 - 4(\sin^2\phi)\, \sigma_1^2\sigma_2^2]^{1/2}\} \tag{5}$$

The probability of being within a $1d_{rms}$ circle varies from 63 to 68 percent depending on the ellipticity of the error ellipse. For a $2d_{rms}$ circle, the probability varies from 95 to 98 percent.

It is common to describe two-dimensional error distributions by two separate one-dimensional standard deviations along each error axis (σ_1 and σ_2). Error circles about a measured position can be constructed for different LOP standard deviations and cut angles. This can be done by first calculating σ_x and σ_y (the standard deviations along the major and minor axes of the error ellipse) knowing the crossing angle and the standard deviations in each measurement (σ_1 and σ_2). Table B-2 is then used to provide probabilities as a function of c, the ratio of the smaller to larger of σ_x and σ_y, and K as the ratio of the error circle radius to the larger of σ_x or σ_y. For example, for a 50° angle of cut, a σ_1 = 15 m (49 ft) and σ_2 = 20 m (66 ft), the probability of location within a circle of 30 m (98 ft) is:

$(\sigma_1)^2 = 225\ m^2$
$(\sigma_2)^2 = 400\ m^2$
$\sin^2\phi = 0.5868$

Substituting into equations 4 and 5, σ_x is 29.9 m (98.1 ft) and σ_y is 13.1 m (43.0 ft).



SOURCE: BOWDITCH 1984

Figure B-3. Illustration of radial error.

TABLE B-2. CIRCULAR ERROR PROBABILITIES AS A FUNCTION OF MEASUREMENT STANDARD DEVIATION RATIOS AND ERROR CIRCLE RADIUS

K \ c	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
0.1	0796557	0443987	0242119	0164176	0123875	0099377	0082940	0071157	0062299	0055400	0049875
0.2	1585194	1339783	0884533	0628396	0482413	0390193	0327123	0281415	0246824	0219757	0195013
0.3	2358228	2211804	1739300	1318281	1039193	0851535	0719102	0621386	0546598	0487639	0440025
0.4	3108435	3010228	2635181	2139084	1742045	1451808	1237982	1076237	0950326	0850326	0768817
0.5	3829249	3755584	3461790	3003001	2532953	2152850	1857448	1626829	1443941	1296280	1175031
0.6	4514938	4457708	4255605	3846374	3357384	2914682	2548177	2251114	2009797	1811783	1647298
0.7	5160727	5115048	4960683	4633258	4170862	3699305	3280302	2925654	2629373	2381583	2172955
0.8	5762892	5725957	5604457	5349387	4941882	4474207	4025028	3627122	3283453	2999700	2738510
0.9	6318797	6288721	6191354	5993140	5651564	5217998	4759375	4333628	3953279	3620135	3330212
1.0	6826895	6802325	6723586	6568242	6291249	5900953	5461319	5025790	4621421	4257553	3934693
1.1	7286679	7266597	7202682	7079681	6859367	6524489	6116316	5687467	5272462	4887873	4539256
1.2	7695607	7682215	7630305	7532175	7359558	7079973	6714269	6308168	5893494	5498736	5132477
1.3	8063990	8050648	8008554	7929968	7793550	7567265	7249673	6873122	6474394	6079822	5704426
1.4	8384867	8374049	8340018	8277048	8169851	7989288	7720889	7383089	7007900	6623035	6219889
1.5	8663856	8655127	8627728	8577362	8493071	8350816	8129287	7833962	7489500	7122546	6753475
1.6	8904014	8897008	8875060	8834914	8768644	8657559	8478393	8226246	7917194	7574708	7219627
1.7	9108691	9103102	9085619	9053766	9001746	8915536	8773116	8562471	8291137	7977592	7642539
1.8	9281394	9276964	9263125	9237989	9197275	9130680	9019110	8846624	8613238	8332175	8021013
1.9	9425669	9422182	9411299	9391586	9359855	9308615	9222277	9083609	8886731	8639149	8355255
2.0	9544997	9542272	9533775	9518415	9493815	9454546	9388418	9278799	9115762	8901495	8646647
2.1	9642712	9640598	9634011	9622127	9603170	9573205	9522999	9437868	9305013	9122714	8897495
2.2	9721931	9720304	9715237	9706109	9691597	9668845	9631017	9565522	9459386	9306821	9110794
2.3	9785518	9784275	9780408	9773450	9762419	9745239	9716934	9667306	9583739	9458085	9259946
2.4	9836049	9835108	9832180	9826918	9818594	9805703	9784661	9747495	9682698	9580804	9413652
2.5	9875807	9875100	9872900	9868953	9862720	9853112	9837569	9810035	9760522	9679136	9560031
2.6	9906776	9906249	9904612	9901674	9897045	9889934	9878527	9858331	9821023	9756969	9659525
2.7	9930661	9930271	9929062	9926894	9923483	9918260	9909944	9893268	9867530	9817837	9739798
2.8	9948897	9948612	9947727	9946141	9943649	9939842	9933821	9923249	9902888	9864476	9801[illegible]9
2.9	9962684	9962477	9961834	9960684	9958978	9956126	9951798	9944246	9929482	9900903	9850792
3.0	9973002	9972853	9972391	9971564	9970266	9968294	9965205	9959854	9949274	9927925	9859910
3.1	9980648	9980542	9980212	9979622	9978699	9977296	9975109	9971348	9963851	9948169	9918113
3.2	9986257	9986182	9985949	9985533	9984880	9983592	9982336	9979733	9974479	9963105	9940240
3.3	9990332	9990279	9990116	9989824	9989368	9988677	9987607	9985792	9982147	9974004	9956522
3.4	9993261	9993225	9993112	9992909	9992593	9992115	9991376	9990129	9987626	9981568	9969113
3.5	9995347	9995323	9995245	9995105	9994888	9994559	9994053	9993204	9991502	9987450	9978125
3.6	9996818	9996801	9996748	9996653	9996505	9996281	9995938	9995364	9994218	9991442	9984662
3.7	9997844	9997832	9997797	9997733	9997633	9997482	9997251	9996867	9996102	9994208	9989352
3.8	9998553	9998545	9998522	9998478	9998412	9998311	9998157	9997902	9997396	9996119	9992842
3.9	9999038	9999033	9999018	9999989	9999943	9999875	9999778	9999608	9999276	9997426	9995020
4.0	9999367	9999363	9999353	9999334	9999305	9999261	9999195	9999085	9998870	9998309	9996[illegible]
4.1	9999587	9999585	9999578	9999566	9999547	9999519	9999475	9999404	9999266	9998900	9997763
4.2	9999733	9999732	9999727	9999720	9999707	9999689	9999661	9999616	9999527	9999292	9998[illegible]23
4.3	9999829	9999828	9999826	9999821	9999813	9999801	9999783	9999754	9999698	9999548	9999034
4.4	9999892	9999891	9999889	9999886	9999881	9999874	9999863	9999845	9999809	9999715	9999475
4.5	9999932	9999932	9999931	9999929	9999925	9999921	9999914	9999902	9999881	9999522	9999[illegible]90
4.6	9999958	9999957	9999957	9999955	9999954	9999951	9999947	9999939	9999926	9999889	9999746
4.7	9999974	9999974	9999973	9999973	9999971	9999970	9999967	9999963	9999955	9999932	9999840
4.8	9999984	9999984	9999984	9999983	9999983	9999982	9999980	9999977	9999972	9999959	9999901
4.9	9999990	9999990	9999990	9999990	9999990	9999989	9999988	9999986	9999983	9999975	9999939
5.0	9999994	9999994	9999994	9999994	9999994	9999993	9999993	9999992	9999990	9999985	9999963
5.1	9999997	9999997	9999997	9999996	9999996	9999996	9999996	9999995	9999994	9999991	9999979
5.2	9999998	9999998	9999998	9999998	9999998	9999998	9999998	9999997	9999997	9999995	9999947
5.3	9999999	9999999	9999999	9999999	9999999	9999999	9999999	9999998	9999998	9999997	9999992
5.4	9999999	9999999	9999999	9999999	9999999	9999999	9999999	9999999	9999999	9999998	9999995
5.5	1.0000000	1.0000000	1.0000000	1.0000000	1.0000000	1.0000000	1.0000000	9999999	9999999	9999999	9999997
5.6								1.0000000	1.0000000	9999999	9999998
5.7										1.0000000	9999999
5.8											1.0000000
5.9											
6.0											

Reference: Bowditch 1984.

Therefore:

$$c = 13.1/29.9 = 0.44$$

and

$$K = 30/29.9 = 1.003$$

Entering Table B-2 for values K = 1.0 and c = 0.4 gives a probability of 62 percent.

Equipment manufacturers quote accuracy probabilities using various terminology. Most commonly, CEP and d_{rms} values are provided. For equal standard deviations ($\sigma_x/\sigma_y = 1$), the d_{rms} is 1.2 times the CEP. Thus, quoting the CEP would be more advantageous to a manufacturer than quoting a d_{rms} value. However, when system accuracy is given as 100 m (328 ft) at the 2σ level, two clarifications are needed: 1) whether absolute or repeatable accuracy is being quoted, and 2) whether measures linear or circular error. If the error is circular, the number describes an 86 percent probability level, whereas if a $2d_{rms}$ is referred to, then the probability of the positioning being within the circle is between 95 and 98 percent, depending on error ellipse axis lengths. Therefore, unless the basis of an accuracy figure is very explicitly stated, it may be necessary to clarify its meaning. Only then can the accuracy performance of competitive systems be compared.

The above discussion is concerned only with the accuracy of the positioning method. However, the drift of sampling equipment away from the vessel (which is usually manifested as a wire angle) will affect the size of the area from which the sample may have been collected. The distance the equipment may drift is, in effect, added to the radial error of the position fix. The increase in the probable area above that inherent in the positioning method is demonstrated in Figure B-4. This larger area of probable sample location is the total error associated with the location of a sampling station, and should be considered by the user in determining if the achievable accuracy is adequate for the purpose of the study.

The increase in the probable sampling location will be a function of both the wire angle and the depth of the station. The increase of the sampling area with depth at various wire angles is exhibited in Figure B-5. The wire angle or equipment drift may not always be noticed or avoidable. A 5 degree wire angle is not uncommon in shipboard sampling and anything less than 2 to 3 degrees may not be noticeable. Wire angle may not be avoidable and the worst-case that may have to be accepted during a study should be used in determining the probable sampling area. The probable sampling areas in Figure B-5 have been calculated by adding wire angle errors to the radial error (calculated from Equation 2) for $\pm$2-m ($\pm$6.6-ft) accuracy positioning methods.

The accuracy of the positioning system chosen also will affect the probable area of sample collection. The less accurate the navigation method,

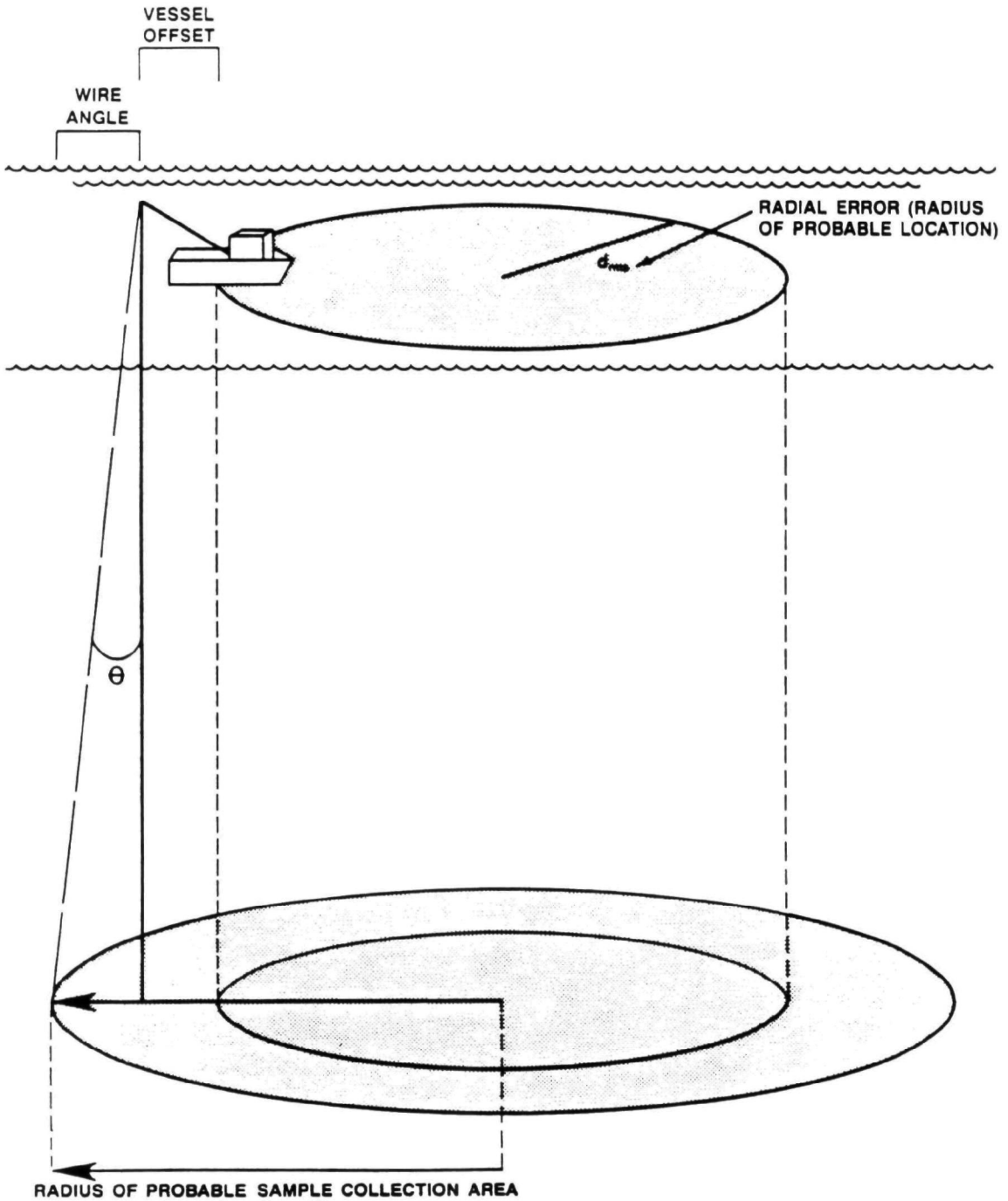


Figure B-4. Components of error in probable area of sample collection.

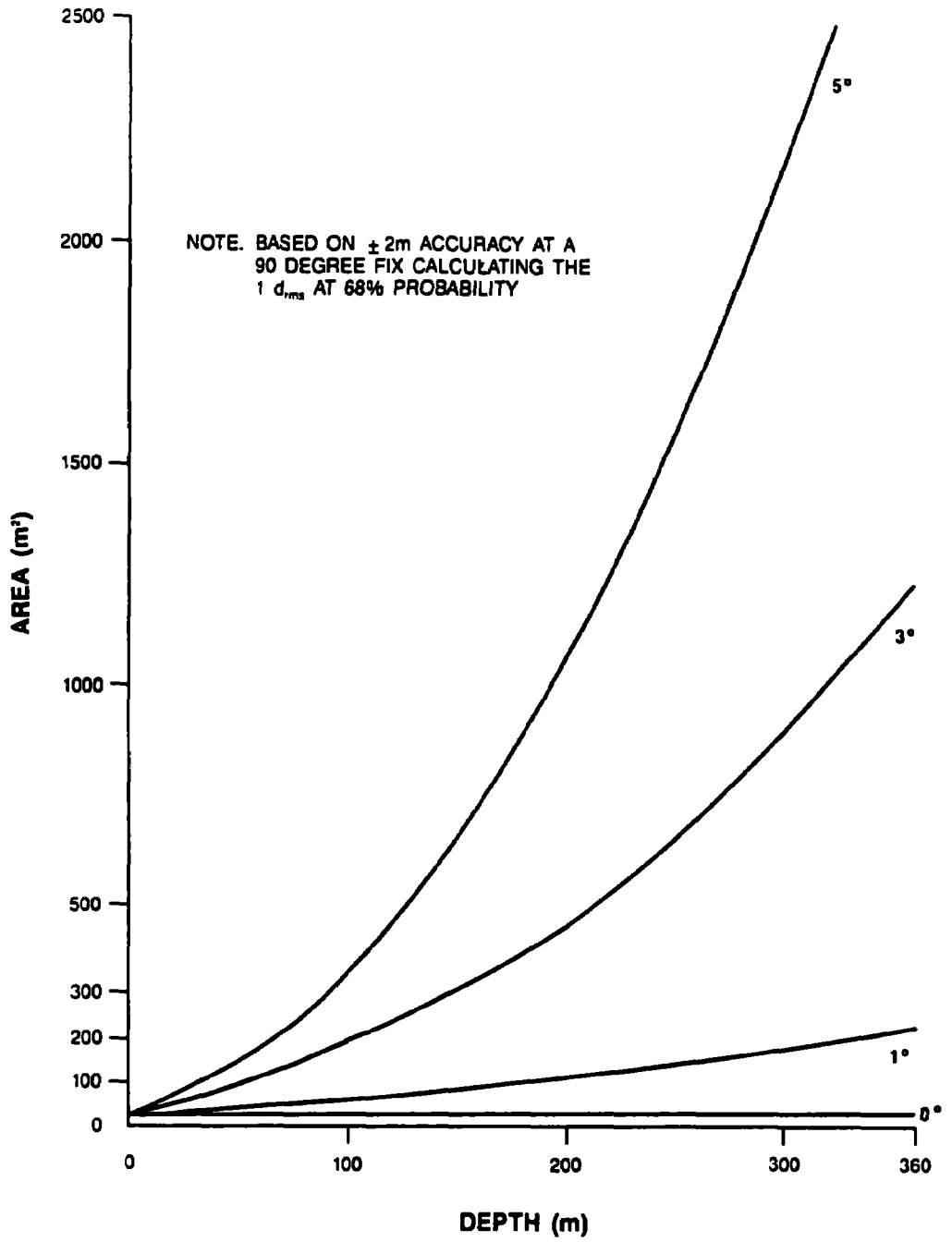


Figure B-5. Effects of wire angle on the probable (P = 0.68) area of sample collection.

the greater the resultant area potentially sampled. The effect of depth on the probable sampling area as a function of the accuracy of the positioning method is presented in Figure B-6. The 5 degree wire angle increases the probable sampling area significantly at depths below 100 m (328 ft).

To ensure that the preferred positioning method will meet all study goals, the error associated with sample collection at specific stations should be estimated. The achievable accuracy can be determined by the radial error utilizing the crossing angle and standard deviations of the LOPs expected over the entire study area. Either Equation 2 or 3 can be used to calculate radial errors at specific probabilities. Tables B-1 or B-2 can be used to determine probabilities associated with a specific radial error. The wire angle error component is then added to the radial error. The distance added to the radial error (see Figure B-4) can be calculated by:

$$d = z(\tan \theta) \tag{6}$$

where: z = the station depth
θ = the wire angle.

A 5 degree angle may be unavoidable and should always be used to calculate the wire angle error component. Substituting into Equation 6 gives:

$$d = 0.09(z) \tag{7}$$

This distance, added to the radial error, will determine the probable area of sample collection for any station.

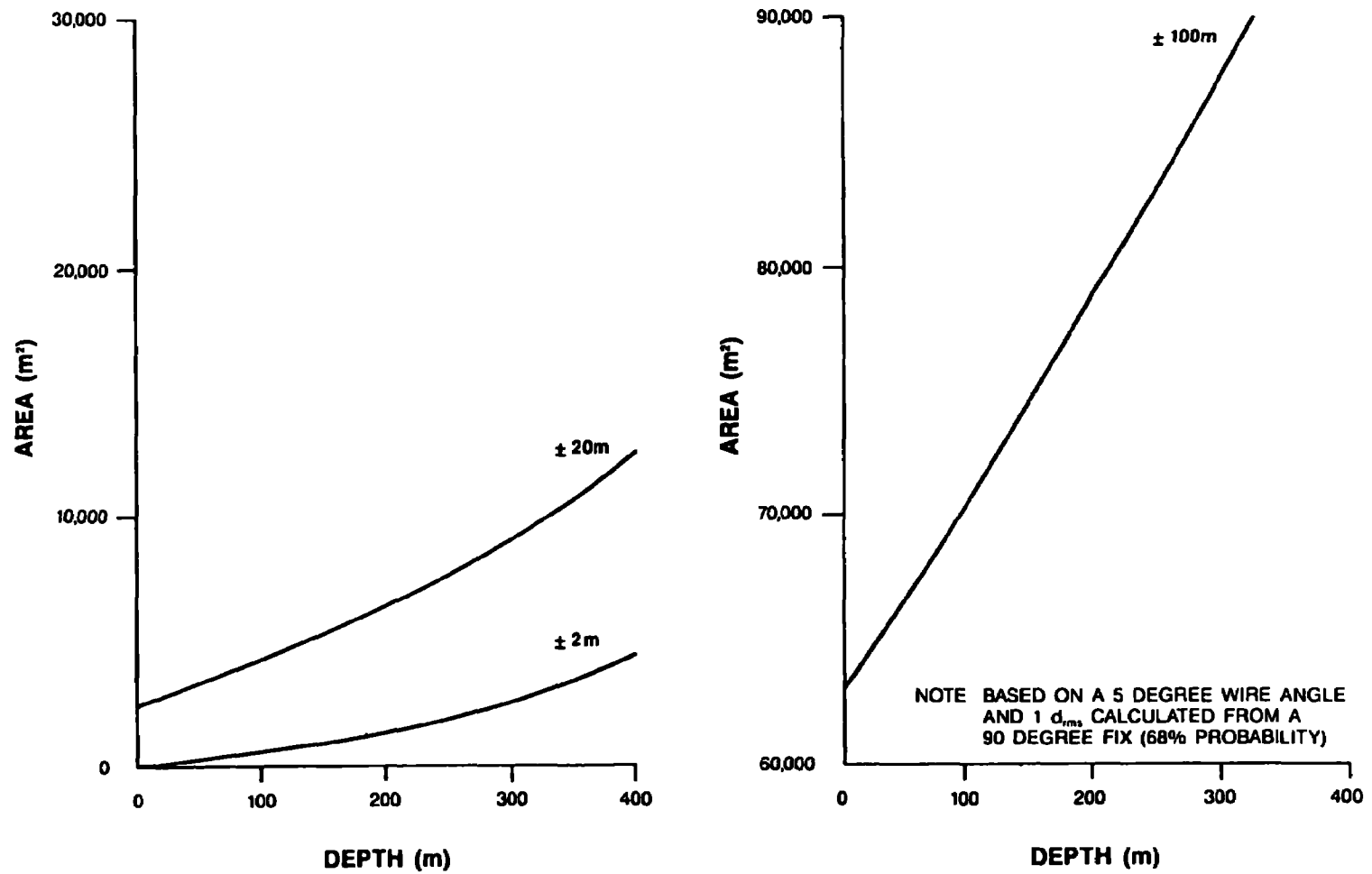


Figure B-6. Effects of navigational accuracy on the probable (P = 0.68) area of sample collection.

APPENDIX B

REFERENCES

Bowditch, N. 1984. American practical navigator. An epitome of navigation. Defense Mapping Agency Hydrographic/Topographic Center, Washington, DC.

Mikhail, E.M., and G. Gracie. 1981. Analysis and adjustment of survey measurements. Van Nostrand Reinhold Co., New York, NY. 340 pp.

APPENDIX C

POSITIONING METHODS AND CONSIDERATIONS FOR SAMPLING IN PUGET SOUND

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POSITIONING METHODS AND CONSIDERATIONS FOR SAMPLING IN PUGET SOUND

The various navigational positioning techniques are listed in Table C-1. This information provides quick-reference review of the performance characteristics and costs of methods representative of each class. These methods are grouped by maximum range to facilitate user comparison for the appropriate sampling sites within Puget Sound. Optical methods are presented first, followed by electronic systems.

OPTICAL POSITIONING TECHNIQUES

Methods Available

The traditional optical positioning method involves observations of two horizontal sextant angles between three fixed shore targets, plotted as a graphical resection using a three-arm protractor or station pointer. Other optical positioning methods are available (Table C-1), but only this and the theodolite intersection method are practical in more open waters [150 m (492 ft) to 5 km (3.1 mi) from shore]. Because the remaining optical methods cited apply only to river or harbor surveys, at extremely short ranges, or in very calm water, they are not useful over the range of expected conditions of sampling in Puget Sound and are only briefly discussed below.

When sampling stations are located relatively close to shore in industrial areas, they can be identified by relatively inexpensive methods. Use of a graduated tape or wire is impractical except in the case of very short distances offshore. Intersecting ranges are used when a number of established landmarks permit easy selection of multiple ranges that intersect at the desired sampling point, and accuracy is not critical. Range line and uniform speed or angle between the line and an onshore target are more applicable to hydrographic surveys than discrete sampling station locations. Vertical angle ranging requires a graduated shore line target known as a subtense bar. A sextant is set so the individual marks on the bar subtend a given angle at desired distances from shore. This method is limited to extremely confined areas, relatively short distances, and calm waters. The angle and stadia method was used historically in very calm waters, but can be used at longer ranges. This method requires vertical positioning of the stadia (or rod) on the vessel and careful measurement of angles to the stadia hairs, enabling a calculation of distance from the observer to the rod. A position fix is made by measuring the azimuth from a baseline of known orientation.

TABLE C-1. SUMMARY OF VESSEL POSITIONING METHODS

Close Range-Direct [up to 5 km (3.1 mi)]

- Horizontal Sextant Angle Resection
- Theodolite Intersection from Shore
- Subtense Ranging by Vertical Angle
- Intersecting Ranges
- Range Line and Angle from Shore or Vessel
- Angles from Shore and Vessel
- Angles and Stadia or Distance from Shore
- Range Line and Uniform Speed
- Distance Line Ranging

Close Range-Indirect [up to 5 km (3.1 mi)]

- Laser
- Infrared Electromagnetic Distance

Short Range [up to 40 km (25 mi)]

- Variable Range Radar
- Microwave Electronic Positioning (300 MHz-300 GHz)

Medium Range [100-300 km (62-186 mi)]

- Medium and High Frequency Electronic Positioning (300 KHz-300 MHz)

Long Range [to 2000 km (1243 mi)]

- Low Frequency Electronic Positioning (30-300 KHz)

Global Positioning

- Very Low Frequency, Satellite, Astronomical Observations (3-30 KHz)

An optical range finder may be used to establish the predetermined distances to permanent targets. An optical range finder is used by simply focusing a split-image on the target, enabling the slant distances to the target to be read off the instrument scale. When combined with a careful compass reading, this distance reading allows positioning of the sampling vessel. A survey of accuracies claimed for commercially available instruments suggests that ±3 m (9.8 ft) accuracy can be achieved within approximately 100 m (328 ft) of the sighted targets. The Lietz Model 1200, for example, provides an accuracy of ±1 m (3.3 ft) at 100 m (328 ft). Beyond this distance, instrumental errors increase rapidly. For the instrument cited, a ±9 m (29.5 ft) accuracy is quoted at 300 m (984 ft). The suggested U.S. list prices of optical range finders vary from $35 to $120 (Folk, L., personal communication). Accuracies are affected by the availability of targets of known position and compass readings. Both of these errors are hard to quantify, making this method unpredictable and of limited usefulness.

Visual lines have sometimes been used to establish a station position. This method requires that a minimum of two objects are in alignment, enabling the vessel to be placed on a common axis extending to the vessel's position. Simultaneous sighting on a second set of at least two objects places the vessel at the intersection of the two common axes. The accuracy of each visual line is highly dependent on the quality of the visual range (e.g., alignment), the distance from the alignment objects to the vessel, and the angle between each range. Also, the number of visual fixes used affects the magnitude of the positional error. Although this technique is frequently used for positioning single sampling stations in bays and harbors or otherwise where a number of convenient alignable targets can be selected, the method has limitations for large scale monitoring programs. Even when a desired sampling station has been accurately located using one of the previously discussed methods, and at that location at least three aligned target-pairs are visible, it not likely that sufficient alignment target-pairs will be present for all other desired locations. Also, the unpredictability of positional error each time a station is reoccupied detracts from the value of this method.

Because the techniques described here are inexpensive to implement (as are use of the sextant resection of theodolite intersection methods), they are attractive to small studies. However, use of more sophisticated and less labor-dependent techniques may be achievable at moderate costs by renting or leasing, rather than buying such equipment. All such options should be considered, provided the recommended absolute positional accuracies can be achieved by the technique selected.

Theodolite Intersection

Position of the sampling vessel can be established by two onshore observers who simultaneously measure the angle between a reference object or shore traverse and the vessel (Figure C-1). A rod or other aiming point is normally erected on the vessel. Radios, flags, or lights signal the

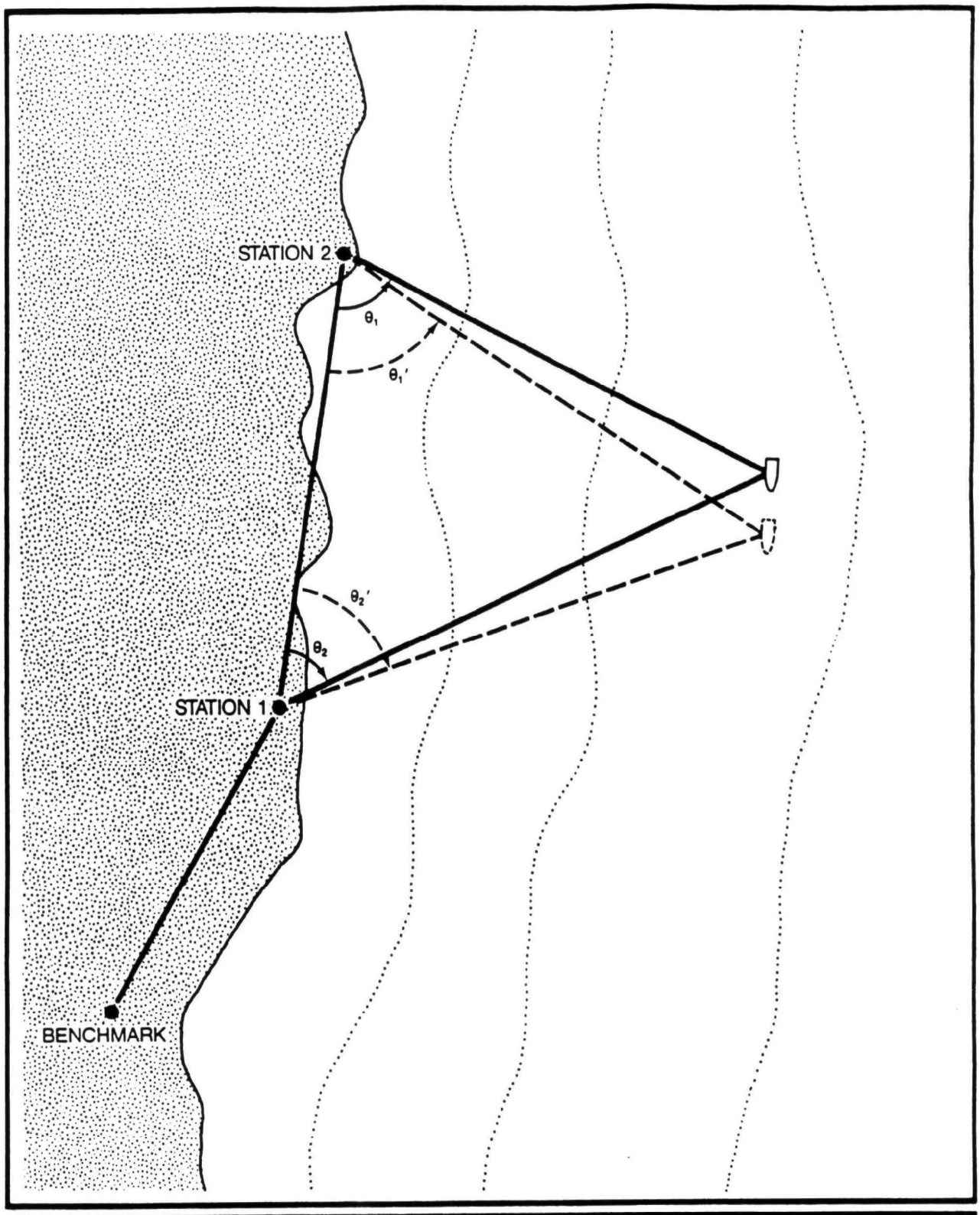


Figure C-1. Station positioning by theodolite intersection.

moment at which angle measurements should be made. A theodolite with an accuracy of ±15 sec for single angle measurement, intercept angles near 45°, and a range of 5 km (3.1 mi), should yield a position error less than ±1 m (3.3 ft) (Ingham 1975). Characteristics of theodolites used for such measurements are summarized in Tables C-2 and C-3.

Although accuracy of this method appears high, its use in open waters has several distinct disadvantages. Complex arrangements usually are needed to ensure that angles are measured by two onshore observers at the same instant of the desired fix. Though not a problem when the vessel remains on station for a long period of time, it becomes a problem when the vessel is trawling. Lines from the two theodolites should intersect at nearly right angles. As indicated in the error analysis discussion, weak position fixes or corresponding large positional areas of uncertainty result when the angles measured are less than 30° or more than 150°. To avoid such extreme angles, onshore observers may have to move frequently, which can delay a predetermined sampling schedule. Each of the onshore observer stations must be surveyed to maintain accuracy. Finally, as with all optical methods, target movement and path interferences (e.g., fog, heavy rain, or heat waves) can confound the measurements. In spite of these disadvantages, the procedure offers relatively high accuracies at low capital cost (although labor cost can rapidly add up) and has been successfully applied in small-scale coastal surveys during favorable weather. It also is advantageous in very restrictive areas or in harbors. It is therefore considered a candidate method for very limited monitoring programs or surveys.

Sextant Angle Resection

An offshore position can be fixed by measuring the two horizontal angles between lines-of-sight to three identifiable targets with known positions. When a vessel is underway, the sextant angles must be measured simultaneously by two observers. The measurement of the first angle between the center and one outside target allows determination of a circle of position (COP) on which the sampling vessel must lie (Figure C-2). For example, when the measured angle is 52°, the first circle of position is plotted by subtracting this angle from 90° and drawing lines seaward from the siting targets at the resultant angle of 38° from the baseline. These lines cross at the center of the COP, which can then be drawn with a compass. This procedure is repeated using the center and remaining targets, resulting in the plot of a second COP. In the example, the second angle is 67°, requiring a plot of radius lines at 23° from the baseline. The intersection of the two position circles marks the vessel's location.

Position fixes are normally plotted with a station pointer or a three-arm protractor (Figure C-3). The two measured angles are set by moving and locking the two outer arms of the protractor, which then is moved over a nautical chart until the three arms align with the preplotted locations of the shore targets. The vessel's position is recorded at the center of the protractor. Because this procedure can be implemented in 10-15

TABLE C-2. SUMMARY OF VERNIER TRANSIT AND SCALE-READING THEODOLITE CHARACTERISTICS

COMPANY	MODEL	VERNIER MICROMETER OR SCALE DIVISION	POSSIBLE ESTIMATION	U.S. SUGGESTED LIST PRICE*
Benchmark	JENA 020	20"	10"	$2495
Berger	Bronze 65/45	20"/1'	20"/30"	$2125/3850
	Aston 67	20"	20"	$1835
	Project 100	1'	30"	$1100
	ST-1/6	1'/20"	30"/20"	$699/1499
	ST-8/9	20"	10"	$1699/1899
Kern	K1-S/ST	30"	6"	$3895/3995
Leitz	BT20/10C	20"	10"	$1695/1995
	115	1'	30"	$795
	T520A/S6	1'	20"/6"	$2495/3695
Nikon	NT-2S MK III	1'	0.2'	$3195
	Schneider 700/400	20"/1'	6"/20"	$1235/650
Pentax	GT-4B/6B	20"		$1695/1895
	TH-60S/60E	1'	6"	$2500/1895
Teledyne	OP 107/100-A-20	20"	20"	$1295/1185
	G-15	20"	20"	$1350
	400W	1'		$650
Topcon	AG-30B	30"	15"	$1595
	TL-60SE	1'	20"	$2100
Warren-Knight	10-2220/3200	20"/1'	10"/30"	$2695/1295
White	TR-300	1'		$749
	TR-303/303PM	20"		$1879/1995
	T-307AT/309T	1'/20"	6"/10"	$2695/2350
Wild	T-16		12"	$1895
	T-05	20"	10"	$3950
Zeiss	Th-42/43	20"	10"	$3950

*January 1985

TABLE C-3. SUMMARY OF MICROMETER AND DIGITIZED THEODOLITE CHARACTERISTICS

COMPANY	MODEL	VERNIER MICROMETER OR SCALE DIVISION	POSSIBLE ESTIMATION	U.S. SUGGESTED LIST PRICE*
Benchmark	JENA 010A	1"	0.1"	$4295
	JENA 015B	6"	1"	$3495
Kern	DKM2-A/T	1"	0.1"	$5890
	DKM3/DKM3-A	0.5"	0.1"	$12295
Lietz	DT-20E	20" Accuracy	10" Display	$2995
	TM6/10E/20H	6"/10"/20"	2"/5"/10"	$3995/3695/3295
	TM-1A	1"	0.1"	$5995
Nikon	NT-1/5A	20"/1"	6"/0.25"	$1895/5950
	NT-4D/3D/2D	6"/10"/20"	1"/2"/4"	$3695/3595/3095
Pentax	TH-20D/10D/06D	20"/10"/6"	5"/2"/1"	$2850/3500/3690
	TH-01W	1"	0.5"	$4900
David White	TH 10-20/10	20"/10"	2"/1"	$2850/3500
	T-308AT/208AT	20"/10"	10"/5"	$2995/3495
	TH 10-1	1"	0.1"	$5500
Topcon	DT-20	LCD Readout 20"	-	$2695
	TL-20DE/10DE/6DE	20"/10"/6"	10"/5"/3"	$2859/3500/3690
Wild	T-0/T-1	20"/6"	20"/3"	$3995
	T-2/T-3	1"/0.2"	0.1"/0.1"	$5995/11995
	T-2000	0.5"	0.1"	$13995
Zeiss	Th-2	1"	-	$5950
	ITh-2	0.6"	-	$16500

*January 1985

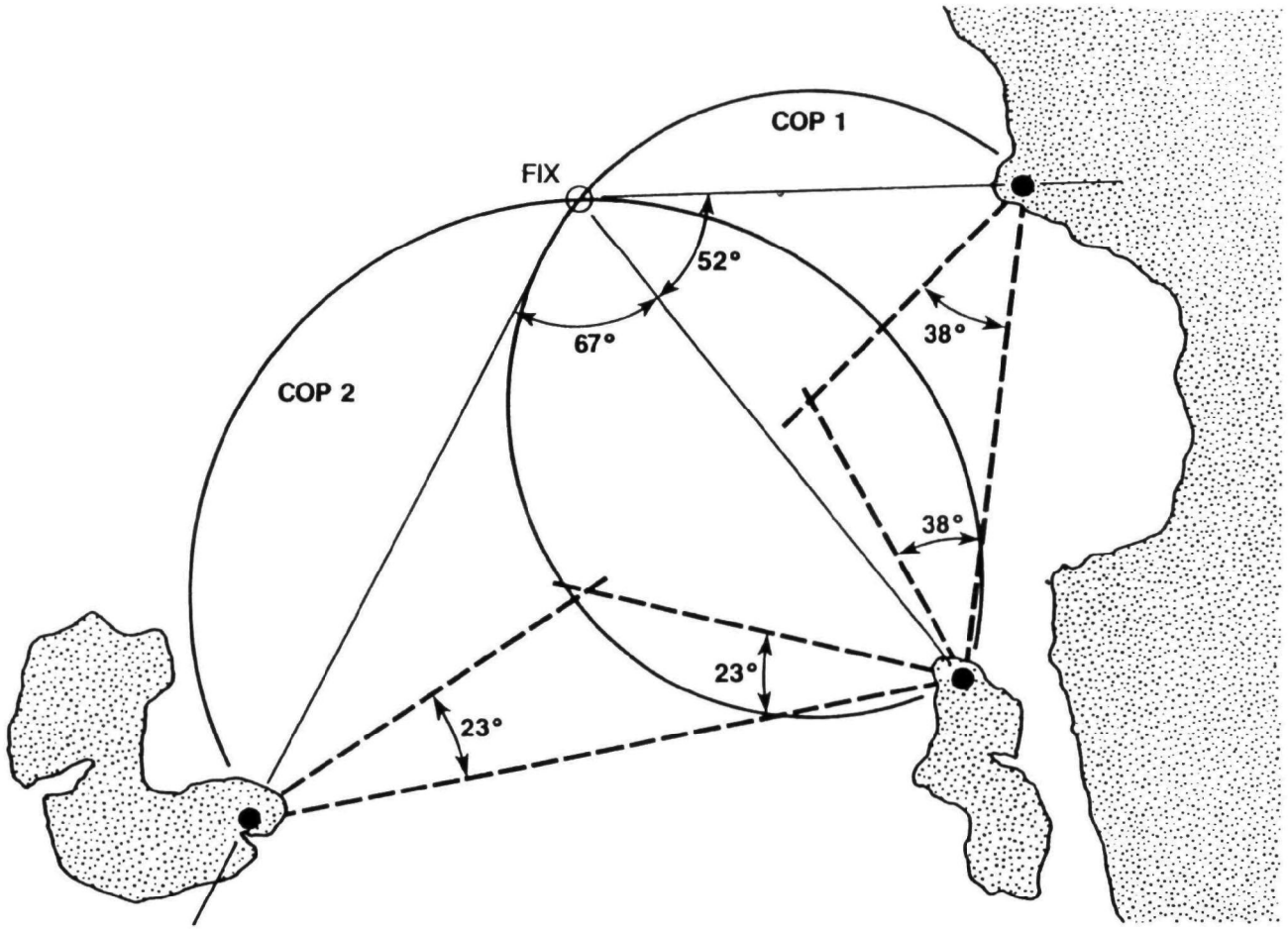


Figure C-2. Station fix using position circles.

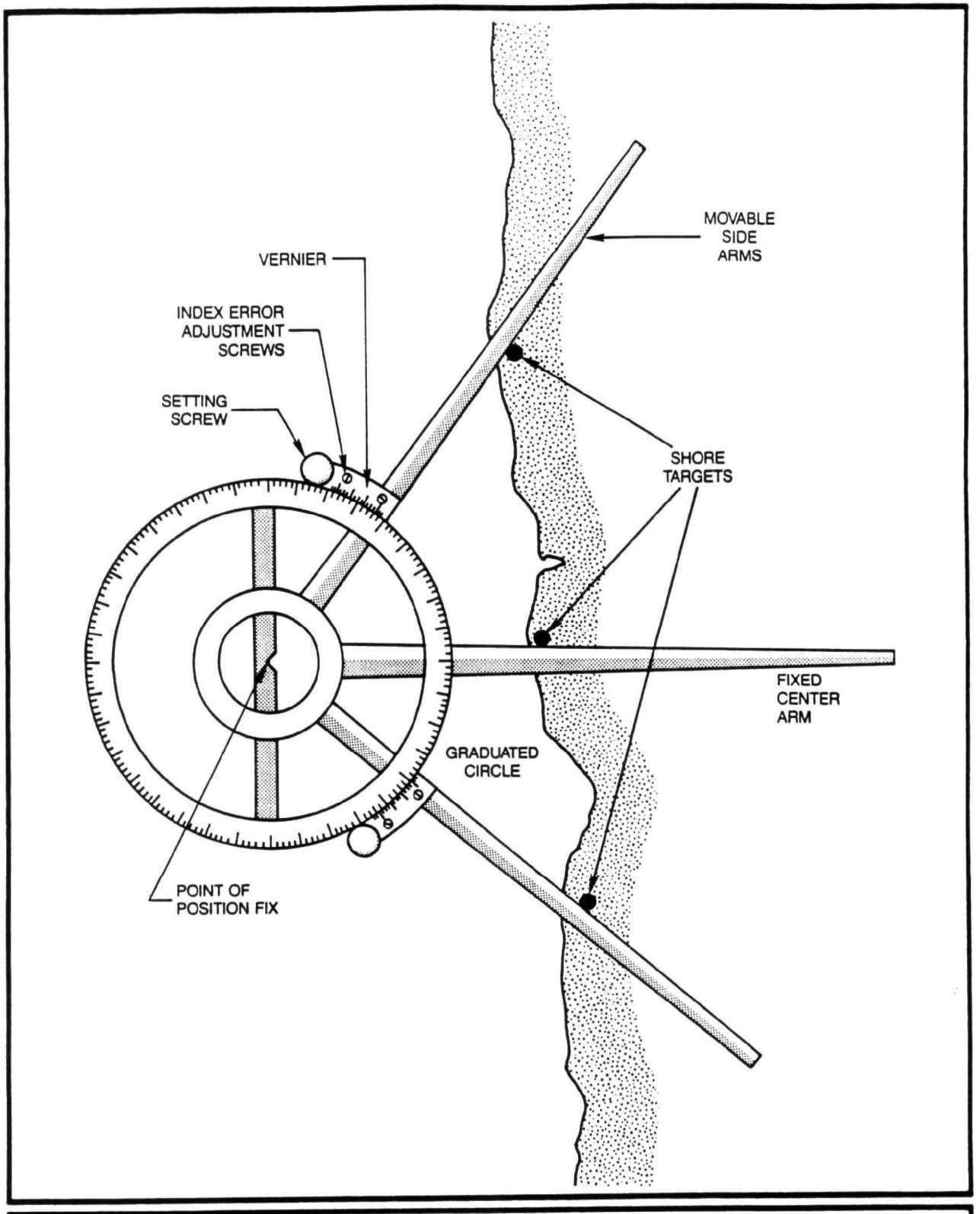


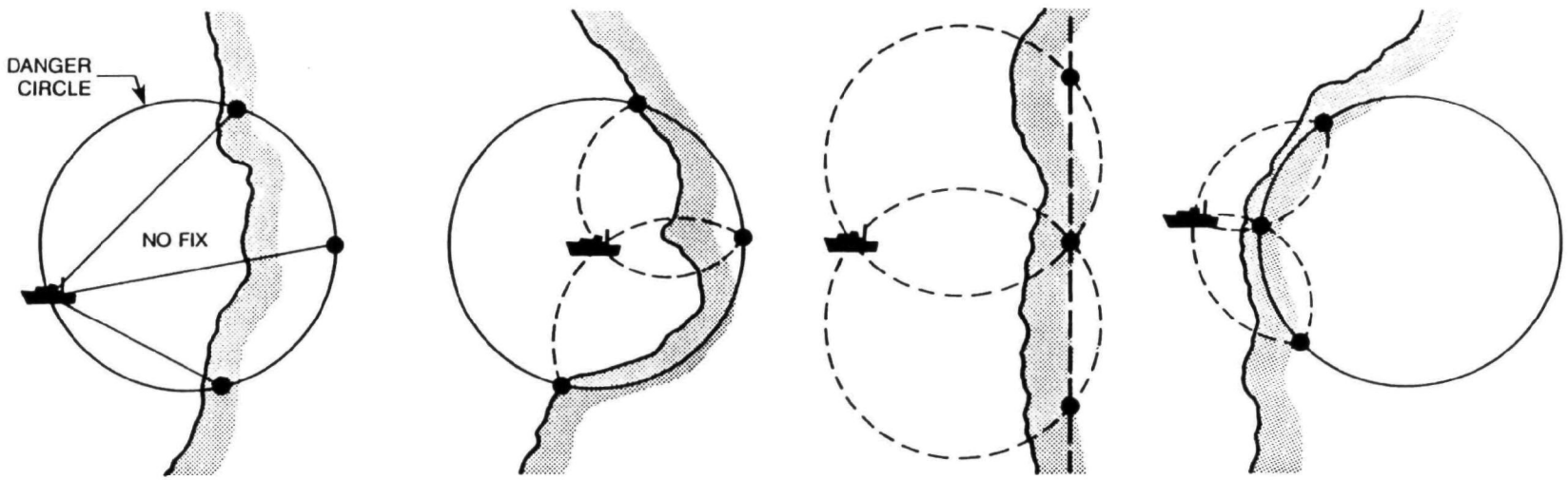
Figure C-3. Three-arm protractor for sextant resections.

sec with some experience, it commonly is used in hydrographic surveys where moving vessel positions are needed. To minimize the parallax error, the two sextant operators should stand as close as possible when making the measurements. Sextant angles can routinely be measured to approximately 1 min of arc or better, depending upon the instrument quality and operator ability. Within 5 km (3.1 mi) of shore stations and at acceptable COP crossing angles, the resulting accuracy in position is 1 part in 2,500, or about ±2 m (6.6 ft) (Ingham 1975).

Sextant angle resection has been the most widely used positioning technique in coastal surveying. This is due to its relatively high accuracy, ease of implementation, and nominal cost of the sextants and the three-arm protractor. Also, no shore party is required. The procedure does have some limiting factors, however. Range is ultimately limited by visibility and by the sizes, elevation, and placement of the shore targets. Also, it is imperative to follow procedures in locating targets to avoid indeterminate or weak fixes. For example, a fix cannot be obtained when the vessel and all three shore targets lie on a common circle (called the danger circle). This can be avoided by aligning the shore targets along a straight line, which causes the radius of the danger circle to become infinite. Another recommended practice to assure strong fixes is to place targets so that intercepted angles fall between 30° and 140° (ideally between 45° and 60°), thereby maintaining large position circle cut angles. Shore targets also may be placed on a curve convex to the observer, with the middle target nearest the sampling vessel (Figure C-4). Alternately, targets may lie on a curve concave to the vessel, provided that the anticipated positions are within the triangle formed by them, they are virtually equidistance from the vessel, the observed angles are not less than 60°, or the vessel is well outside the circumscribing circle (Clark 1951; Davis et al. 1966).

At the limits of visibility, sextant angle fixes are likely to be weak because the angles are small. In such cases, large positional errors can be caused by small errors in the angles themselves (Umbach 1976). This problem can be partially offset by using a telescope mounted on the sextant. Sextants must be in perfect adjustment and angles must be read with extreme care. Also, the sextant must be of superior quality so that the angles may be read to the necessary precision. When working at locations near shore, the sum of the two angles can approach 180°, with one angle often very large and the other very small. Under such conditions, care must be taken to mark the two angles simultaneously if the vessel is moving, or to make several measurements if the vessel is on station because the angles rapidly changed with slight vessel movements.

Split-fixes (no common center object) may be taken when a three-point fix is not possible. The vessel position is at the intersection of two angle loci. A fix is considered strong when the intersection angle is greater than 45°. While shoreline tangents can be taken when no other objects are available these fixes are inaccurate in most cases. Split fixes and shoreline tangents should be used only when absolutely necessary.



Adapted from Ingham 1975

Figure C-4. Shore target locations to avoid the danger circle.

They are inefficient due to the required recording procedures and plotting time, and they cannot generally be entered into automatic data processing and plotting systems.

Sextants are classified as vernier or micrometer drum types, the latter preferred by most users. A well-constructed metal marine sextant is capable of measuring angles with an instrument error not exceeding 10 sec or 0.1 min of arc (Bowditch 1984). However, as indicated earlier, positional error will be highly dependent on operator ability. For accurate work, a sextant having an arc radius of 162 mm (6.4 in) or more should be selected. Characteristics of representative sextants are presented in Table C-4. Sextants must be adjusted prior to the start of the survey and verified at the conclusion or at least once a week, whichever is more frequent. Any index correction should be recorded in the survey log. Procedures for sextant adjustment are provided by Umbach (1976) and Bowditch (1984).

Considering the achievable accuracy when the double horizontal sextant angle method is properly implemented, this procedure offers an inexpensive candidate positioning method for limited surveys. However, the method may be ineffective during poor visibility or periods of heavy weather when monitoring or seasonal survey work may nonetheless be required. Also important is the need to construct, survey, and maintain a sufficient number of properly located shore targets to provide unambiguous positioning. Overall, the method has merit when the cost of an electronic positioning system cannot be justified.

Variable Range Radar

While technically not an optical positioning technique, obtaining ranges from a variable range radar (VRR) consists of two optical estimations by the user (target location and bearing of radar reflection) and will be addressed herein. A position can be fixed by measuring the distances to three targets on the radar screen that can be accurately identified on a map. A third fix will reduce the chance of error and increase accuracy. A variable range marker measures the distance to the object (as identified by its radar reflection). This distance then is drawn with a compass as a line of position (LOP) on the nautical chart. The intersection of the three LOPs marks the vessel's position.

Most commercial vessels are equipped with radar for safety and navigation. Coastal vessel radar usually have ranges from 26 to 116 km (16 to 72 mi). A variable range marker (VRM), whether built-in or added onto the existing on-board radar, removes a large portion of operator error in estimating distances. For positioning at accuracies less than 300 m (984 ft), a VRM almost always is needed. Range accuracies with the VRM are usually 1 to 2 percent of the range scale, or ±25 m (82 ft) at 0.5 km (0.3 mi), the smallest scale. Accuracy decreases as the range scale is increased. Bearing

TABLE C-4. MARINE SEXTANT CHARACTERISTICS

SPECIFICATIONS	C. PLATH NAVSTAR CLASSIC	NAVSTAR PROFESSIONAL	TAMAYA SPICA	TAMAYA JUPITER (UNIVISION)	TAMAYA VENUS	WEEMS& PLATH
Arc Range	-5 to +125°	-5 to +125°	-5 to +125°	-5 to ±125°	-5 to +125°	-5 to +125°
Instrument Accuracy	< ±10"	< ±10"	< ±10"	< ±12"	< 18"	< ±10"
Vernier Scale	0.2'	0.2'	0.2'	0.2'	0.2'	0.2'
Arc Radius (mm)	162	162	162	162	138	162
Telescope	4X40 6X30	4X40	4X40(7°) 7X35(6.5°)	4X40(7°) 7X35(6.5°)	3X26	4X40 6X30
Brightness			100/25	100/25	75	
Index Mirror (mm)	57X41	57X30	57X42	57X42	45X30	57X41
Horizon Mirror Dia. (mm)	57	57X30	57	57	45	57
Illumination	Arc & Drum	Yes	Arc & Drum	Arc & Drum	No	Arc & Drum
Frame Mtl.	Brass/Alloy	Makrolon	Aluminum	Aluminum	Aluminum	Brass
Arc Mtl.	Brass/Alloy	Al. Alloy	Bronze	Bronze	Bronze	Brass
Case	Mahogany	Plastic	Plastic	Plastic	Wood	Mahogany
U.S. Suggested List Price (1/85)	$1270	$965	$919	$715	$585	$1674

accuracy is usually less than 1°. Characteristics of representative VRRs are presented in Table C-5. Generally, analog systems have better resolution than digital systems, but are not as versatile.

Each position fix will rely on the resolution and identification of the radar reflection. Resolution of the target position will change with the range scale. Reflections will depend upon target position and alignment. The location of the reflection is not always easy to identify. Placement of the cursor away from the actual reflection surface will introduce error into the fix. Estimation of the radar target location in relation to the chart's mapped structures is important. Misidentification of a reflection source will result in plotting a position at the wrong coordinate, but will not affect repeatable accuracy if the same target is used. Certain regions, such as sloped headlands and tidal flats, give inaccurate reflection because it is impossible to relate the reflection to a map location. The most accurate radar range fixes are obtained from solid reflections between 0.16 and 6.4 km (0.1 and 4 mi). This keeps the range scale low, resulting in accuracies greater than $\pm$40 m (131 ft) and avoids erroneous readings caused from very close reflections.

Positioning vessels with VRR should provide sufficient increases in accuracy over standard radar for most sampling purposes. Repeatable accuracies can be within $\pm$20 m (66 ft). Position fixes from VRR are still available in almost any type of weather. Most vessels already are equipped with VRR and other radar can add variable range markers for $1,000 to $2,000. The newer digital systems, priced in the $5,000 to $10,000 range, offers multilevel processing for better target pickup and provides map plotting ability directly on the screen.

ELECTRONIC POSITIONING TECHNIQUES

Electronic positioning methods use the transmission of electromagnetic (EM) waves from two or more shore stations and a vessel transmitter to define a vessel's location. The systems are based on the ability to predict variations in EM wave travel velocity as a function of travel path. Position is determined by measuring differences in signal arrival times (range-range mode) or by comparing the phases of received signals to that of the transmitted signal (hyperbolic ranging).

At their respective maximum ranges, electronic positioning methods have higher accuracies than visual methods. Measurements can be obtained regardless of weather and visibility conditions, and operating ranges are typically much greater than for optical methods. Range can be extended to 50-100 km (31-62 mi) simply by elevating antennae until signal attenuation becomes a limiting factor. Shore stations need not be attended, minimizing personnel requirements. Positional readouts are available as distances or coordinates, rather than wavelengths or time delays, and deck units are

TABLE C-5. VARIABLE RANGE RADAR (VRR) SYSTEM CHARACTERISTICS

Systems	Model	Type	Variable Range Markers	Range (km)	Nominal Accuracy Range (m)	Nominal Accuracy Angle	U.S. Suggested List Pricea
DECCA	RD170 VRM/VP3	Digital	1 (add-on)	77	-	±0.5°	$3,990
Racal DECCA Marine	RD170 BT	Digital	1	77	±30 at 0.4 km	±0.5°	$6,000
Redmond, WA							
(206) 885-4713							
Furuno U.S.A.	FR-360 MKII	Analog	add-on	58	±25 at 0.5 km	<±1°	$4,400 without VRM
San Francisco, CA							
(415) 873-9393	FR-810	Digital	2	116	±36 at 0.5 km	<±1°	$7,400
	FR-1011	Analog	1	77	±25 at 0.5 km	<±1°	$8,300
KODEN/SI-TEX	T-100	Digital	1	26	±22 at 0.5 km	±0.5°	$2,200
Norwell, MA							
(617) 871-6223							
Raytheon Marine	3604	Analog	1	58	-	±1°	$4,900
Seattle, WA	3610	Analog	1	116	-	±1°	$5,400
(206) 285-6843							

[a] December, 1985.

usually compatible with data processing and automatic plotting equipment. Position information is continuous, enabling maintenance of a desired location by dynamic positioning or by traversing along a predetermined path.

The short-range systems of primary importance in survey and monitoring programs are compact, lightweight, durable, easily calibrated, and relatively stable. Disadvantages of electronic systems can include cost, particularly for smaller program requirements, the inconvenience of orienting shore and on-board units, vandalism of shore stations, and unknown signal propagation effects (although this should not be a problem over the relatively short distances to survey points in Puget Sound). As discussed later, costs can be minimized by sharing the expense among multiple users, by leasing equipment during survey periods, or by contracting for survey personnel and equipment.

System Classifications

Electronic positioning systems often are classified by range capability, which depends largely upon propagation characteristics of the operating signal. Band width and signal power also influence range capability. Electronic positioning methods are herein categorized as short-range, medium-range, and long-range systems. Although short-range systems are emphasized, other categories also are examined because systems such as Loran-C are frequently used in parts of Puget Sound. Satellite navigation systems also are presented because their prices are declining and capabilities (i.e., coverage and repetitive access) are expected to increase. Categories of electronic positioning systems, including operating frequencies, wavelengths, ranges, and representative commercially available equipment, are listed in Table C-6.

Short-range [0-40 km (0-25 mi)] microwave systems are portable and best suited for use in the range-range mode. Medium-range systems [to 150 km (93 mi)] also are transportable, although components usually are bigger and heavier. They are effective in either the range-range or hyperbolic mode. Long-range [to 2,000 km (1,243 mi)] and global systems transmit from permanently installed, widely dispersed shore stations or satellites for multiuser operation.

Comparative Absolute Accuracies

Although it is difficult to specify the positional accuracies achievable by instruments in each category, some generalizations can be made. Whereas the optical methods discussed can provide accuracies of ±2 m (6.6 ft) for ranges up to 5 km (3.1 mi) offshore, short-range electronic positioning systems may provide accuracies of ±1-3 m (3.3-9.8 ft) for ranges up to 40 km (25 mi) from shore stations. Comparable medium-range system accuracies are ±5.0 m (16 ft) up to 150 km (93 mi). Long-range systems typically have accuracies of ±50-100 m (164-328 ft) within 350 km (217 mi) of shore stations, and more than 100 m (328 ft) at greater ranges.

TABLE C-6. ELECTRONIC POSITIONING SYSTEM CATEGORIES

Category	Range	Representative Systems
Very long range	>2,000 km	OMEGA TRANSIT (NAVSAT)
Very low frequency Satellite		GEOSTAR NAVSTAR GPS SERIES AERO SERVICE GPS
Long range	0-2,000 km	LORAN-C VIEWNAV
Low frequency		LAMBDA
Medium range	0-150 km	SYLEDIS RAYDIST TRAK IV
Medium-high frequency		HYPER-FIX ARGO DM-54 HYDROTRAC
Short range		
Radar	0-100 km	DECCA FURNO U.S.A. KODEN/SI-TEX RAYTHEON MARINE
Microwave	0-40 km	TRISPONDER MINIRANGER MICRO-FIX HYDROFLEX AUTOTAPE AZTRAC POLARFIX ARTEMIS

Operating Modes

There are two principal radio navigation system operating modes: the two-range (or range-range) mode and the hyperbolic mode. Some systems incorporate the advantages of each in a combination mode. These three modes are presented in Figure C-5. In the range-range mode, position fixing is accomplished by measuring the extremely small time intervals required for EM signals to travel from an on-board master transmitter to one or more onshore slave stations, and back (Figure C-5a). For a known propagation velocity, the time interval is converted to a distance (range) from the slave, defining a single circle of position on which the vessel may lie. The intersection of two or more such circles (based on signal returns from two or more slave stations) results in a position fix. This operating mode usually is restricted to a single user, although single side-band techniques have been employed to allow multiuser operation (Ingham 1975). Lane width (distance between points of zero signal phase) remains constant regardless of distance from the system baseline. The lane width resolution does not decrease at increasing ranges from the baseline, as is the case in the hyperbolic positioning mode.

In the hyperbolic mode, the on-board receiver detects the phase difference of signals arriving from multiple shore-based transmitters. Lines of constant phase between master and slave transmitters form a hyperbolic pattern of position lines (Figure C-5b). By measuring the phase difference between arriving signals, the vessel can be located along one of the position hyperbolas. Adding a second master-slave transmitter pair superimposes another hyperbolic pattern, resulting in a grid network with pattern crossings (Figure C-5c). Measurement of the signal phase difference from the second transmitter pair allows vessel positioning on the second pattern, and therefore "unambiguous" location at the applicable grid crossing point. Phase differences usually are resolved to 1/100 of the lane width. Resolution of the hyperbolic system matches that of a range-range system only along the master-slave transmitter baseline. Because of lane widening for increasing range from the baseline, the system resolution decreases with vessel distance from the master-slave transmitter baseline. As indicated earlier, the angle-of-cut of arriving signals also affects the magnitude of position error. In hyperbolic systems, extending the baseline length improves cut-angles of the arriving signals and decreases lane spreading (Ingham 1975).

Short-Range Systems

Electronic Distance-Measuring Instruments--

A position fix is obtainable with two electronic distance-measuring instruments (EDMI), or one EDMI with an angle measurement by theodolite or sextant. Distance-measuring instruments that are either electro-optical (e.g., laser) or electronic (e.g., microwave) are discussed herein. Electro-optical and microwave distance-measuring devices are extensively used in

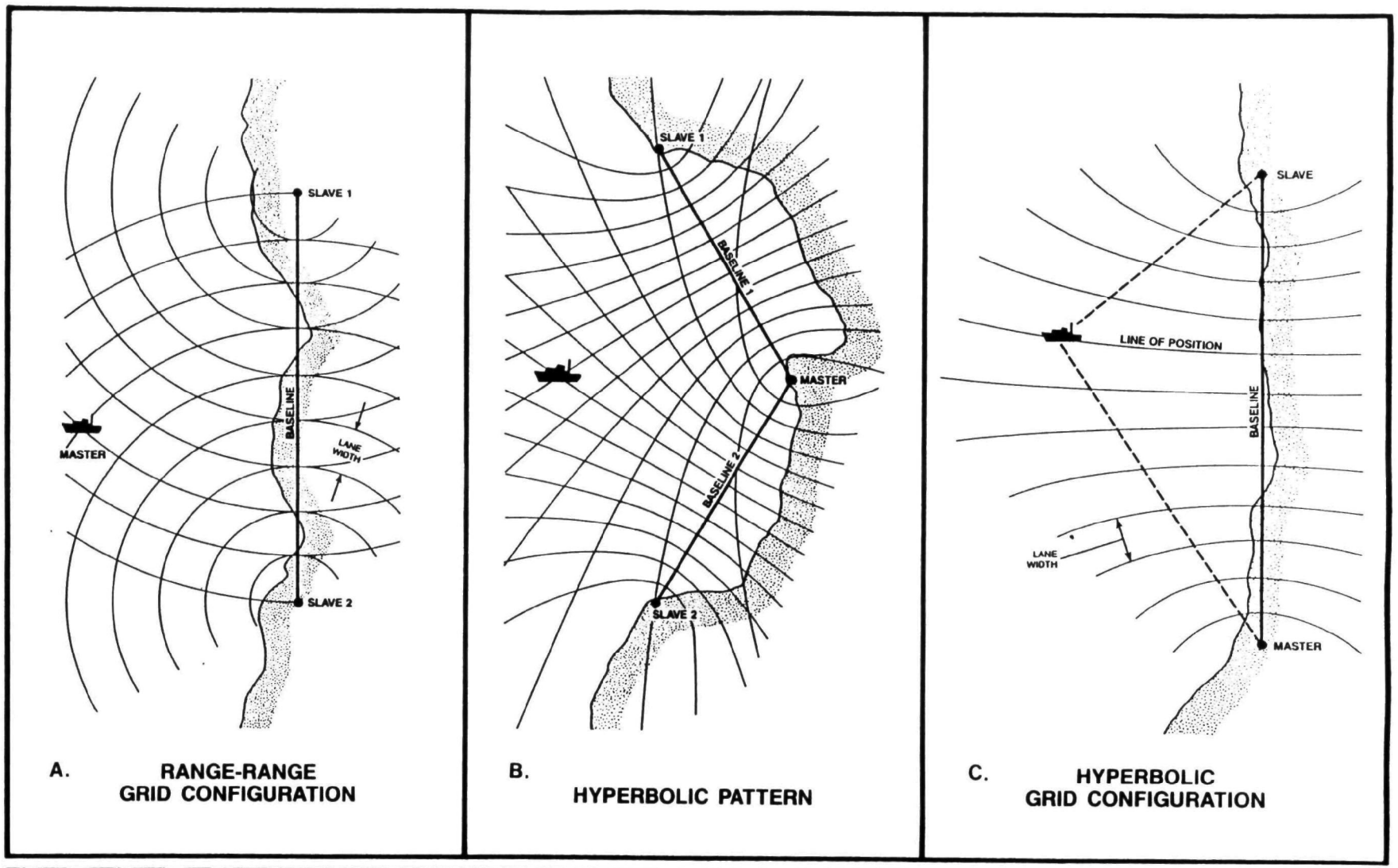


Figure C-5. Operating modes for radio navigation systems.

land-based surveying. The EDMI master generates a carrier signal which is directed toward a reflector (in the case of light beams) or a repeater (for microwaves). The light or microwave beam is modulated at two or three different frequencies, usually under the control of a precision quartz crystal oscillator. A phase comparison of the incoming and outgoing beams enables accurate distance determinations.

The EDMI is relatively new in the surveying field. The geodimeter of the early 1950s, which used a modulated light beam, was replaced in the late 1950s by the Tellurometer, which used a modulated microwave signal. This improvement increased the range and allowed operation in moderate rain, fog, and darkness. Newer EDMIs have shorter ranges, but due to the use of solid state electronics, are much more compact, less power-intensive, and easier to read. The newest EDMIs use highly coherent laser light, have longer ranges, require even less power, are portable, and are easy to operate. The so-called "total station" consists of a theodolite for measuring angles and an EDMI for measuring distances, with outputs recorded on magnetic or paper tape for subsequent analyses. Under favorable conditions, EDMI range capabilities are 1.6 km (1 mi) for light-based systems, 80 km (50 mi) for laser systems, and 150 km (93 mi) for microwave systems.

Properly adjusted and calibrated, an EDMI has few sources of error. Ground wave reflection can cause error when measurements are made over water because reflected signals result in faulty distances due to the longer path lengths. The swing, or cyclic manner, in which reflections are recorded must be correctly interpreted. At very close range, EDMI accuracy is limited by a constant of uncertainty. Beyond 0.5-1.0 km (0.3-0.6 mi), accuracies of 1 part in 25,000 are easily achieved. If meteorological conditions over the signal path are sufficiently well-known, accuracies of 1 part in 100,000 can be achieved (Moffitt and Bouchard 1982). Characteristics of representative short- and long-range electro-optical and microwave distance measuring devices are presented in Table C-7. As indicated, instruments with a range of 25 km (16 mi) can be used to measure distances to within 5 cm (2 in), while shorter-distance devices [to 5 km (3.1 mi)] are accurate to 1.5-3.0 cm (0.6-1.2 in)].

It is apparent, therefore, that accuracies achievable with electronic distance measurement devices are more than adequate to meet the positioning requirements for surveys and monitoring programs. In fact, it is the angle-measuring devices used with EDMIs that limit accuracy, not the EDMI itself. Probably the major disadvantage of EDMIs is the continual motion and resultant misalignment of the reflector (in electro-optical systems). Use of microwave patterns eases directivity requirements.

Total Stations--

An electronic tachymeter, commonly referred to as a total station, is an instrument for determining the distance, bearing, and elevation of a distant object. In coastal surveys, it is a shore station instrument

TABLE C-7. ELECTRONIC DISTANCE MEASURING INSTRUMENTS

COMPANY	MODEL	RANGE(m) SINGLE PRISM TRIPLE PRISM MAXIMUM(prisms)	ACCURACY (MSE)	U.S. SUGGESTED LIST PRICE
Benchmark Orlando, FL (305)281-5000	Surveyor III-X	1600 3000 3500(6)	±(5 mm + 5 ppm)	$3,495
Geodimeter, Inc. Novato, CA (415)677-1256	Geodimeter 14-A	6000 8000 15000	±(5 mm + 3 ppm)	$11,300
	Geodimeter 112/122	2500 3600 6000(8/16)	±(5 mm + 3 ppm)	$6,250(112) $10,950(122)
	Geodimeter 220	1600 2400 3200(8)	±(5 mm + 3 ppm)	$8,850
Kern Instruments Brewster, NY (914)279-5095	DM 503	2000 3500 4500(7)	±(3 mm + 2 ppm)	$8,995
Keuffel & Esser, Co. Morristown, NJ (201)285-5000	Ranger V-A (HeNe Laser)	8000 16000 25000	±(5 mm + 2 ppm)	$20,561
	PulseRanger	1000 3000	±(30 cm + 150 ppm)	$7,500
The Lietz Company Overland Park, KS (913)492-4900	RED 2A/2L	2000/3800 2800/5000 ----/7000(9)	±(5 mm + 5 ppm)	$4,695(2A) $5,795(2L)
MK Electronics, Inc. Littleton, CO (303)795-2060	MK-III, MK-III VS	1600 3000 4000	±(5 mm + 2 ppm)	$5,950(III) $7,950(III-VS)
Nikon, Inc. Garden City, NY (516)222-0200	ND 31	1900 3200	±(5 mm + 5 ppm)	$5,885
Pentax, Corp. Englewood, Co (303)733-1101	PM-81	1400 2000	±(5 mm + 5 ppm)	$4,790
Teludist, Inc. Mastic Beach, NY (516)399-5843	Tellemat CMW20 (microwave)	25000	±(5 mm + 3 ppm)	$16,500
Topcon Instrument Corp. Paramus, NJ (201)261-9450	DM-S3	2000 2500 2900(9)	±(5 mm + 5 ppm)	$5,390
Wild Heerbrugg Farmingdale, NY (516)293-7400	Citation-450	1600 2300 4000(11)	±(5 mm + 5 ppm)	$3,995
	D1-4L Distomat	2500 3500 7000(11)	±(5 mm + 5 ppm)	$8,995
	D1-20 Distomat	6000 7000 14000(11)	±(3 mm + 1 ppm)	$14,995

used to sight the survey vessel reflectors, enabling positional information to be recorded onshore for subsequent communication to the vessel operator. In a manual station, the same telescope optics (co-axial) are used to measure both distance and angles. They basically are theodolites with built-in EDMI units. With such manually operated units, slope reduction of distances is done by optically reading the vertical angle and keying it into a built-in or hand-held calculator (McDonnell, Jr. 1983). A semiautomatic total station contains a vertical angle sensor for automatic slope reduction of distances, while horizontal angles are optically read. With an automatic station, both horizontal and vertical angles are electronically read for use with slope distances in a data collector or internal computer. A theodolite with a mount-on EDMI usually is not classified as a total station. An exception is the modular total station, where the design objective is flexibility of future additional equipment. Such units usually are designed around an electronic (digitized) theodolite such as the Kern E1. Many total stations are designed to make full use of hand-held calculators (e.g., the HP-41CV) for data storage, computations, access to control registers, testing, calibration, and orthogonal offset determinations. Most manufacturers offer optional data collectors that serve as electronic supplements to field books. This permits a convenient interface with a computer and remote transmission of data using an acoustic modem.

Characteristics of representative total stations are presented in Table C-8. As indicated, range is dependent upon the number of prisms available for signal reflection. Such prisms are directional (i.e., must be pointed towards the shore station), as opposed to omnidirectional prism arrangements used for range-azimuth navigation systems. Directional prisms cost approximately $300 for a pair and $500 for a set of three. Although systems requiring 9 to 11 prisms are expensive, they allow measurements regardless of vessel orientation. Some manufacturers report displaying angles or accuracies to 1 sec or less. However, experienced surveyors know that this level of accuracy requires careful or repeated pointings at good targets.

For surveys and monitoring programs, single station capability is attractive. Setup and calibration efforts are minimized, and the logistics of station movement are much simpler than with a multi-station net. A total station can be used on other projects when not in use for periodic monitoring. The $8,000 to $30,000 price range is competitive with the microwave positioning systems ($40,000 to $100,000), and achievable accuracies in both range and angle are more than adequate. Instrument capabilities and costs are reported in the free, bimonthly journal Point of Beginning [P.O.B. Publishing Company, Wayne, MI (313-729-8400)].

Microwave Navigation Systems--

Short-range electronic positioning systems generally operate at microwave frequencies that limit the system ranges to "radio line-of-sight." Typically, such systems are effective between 25 and 100 km (16 and 62 mi) offshore,

TABLE C-8. TOTAL STATION CHARACTERISTICS

COMPANY	MODEL	TYPE	RANGE* (km) L	RANGE* (km) M	RANGE* (km) H	PRISMS	ACCURACY RANGE	ACCURACY ANGLE	U.S. SUGGESTED LIST PRICE
Carl Zeiss, Inc.	RMS3	Semi-Auto	-	1.5[a]	-	1	±5-10 mm + 2ppm	±2"	$8,260
Thornwood, NY			-	2.0[b]	-	3			
(914)848-1800			-	3.0[c]	-	9			
	Elta 3	Automatic	-	1.6	-	3	±10 mm + 2ppm	±2"	$18,725
			-	2.5	-	6			
			-	3.0	-	18			
	Elta 46R	Automatic	-	2.0	-	3	±10 mm + 2ppm	±3"	$12,820
Geodimeter, Inc.	140	Automatic	1.2	2.2	3.0	1	±(5mm + 5 ppm)	±2"	$19,950
Novato, CA			1.8	3.0	4.0	3			
(415)883-2367			2.4	3.8	5.5	6			
			3.6	4.8	6.0	8			
Kern Instruments	E1/DM503	Automatic	1.5	2.5	3.0	1	±(3 mm + 2 ppm)	±2"	$19,175
Brewster, NY	E2/DM503		2.0	3.5	4.5	3	±(3 mm + 2 ppm)	±0.5"	$22,375
(914)279-5095			2.4	4.5	5.5	7			
'etz	SMD-3	Manual	-	0.8	1.2	1	±(5 mm + 5 ppm)	10" digital	$8,800
ɔrland Park, KS	SDM-3ER	Semi-Auto	-	1.4	1.8	3		5" direct	$10,800
,13)492-4900	SET-10	Automatic	-	-	2.5+	9			$14,000
MK electronics	MK-III	Automatic	-	3.0	-	3	±(5 mm + 2 ppm)	6"	$12,950
Littleton, CO	MK-IV		-	4.0	-	3	Stationary		$14,950
(303)795-2060	MK-HYDRO		-	3 or 4	-	3	±(20 mm + 5 ppm) Moving		$16,950
Nikon Instruments	NTD-4	Manual	-	1.2	1.6	1	±(5 mm + 5 ppm)	6" digital	$8,595
Garden City, NJ			-	1.8	2.3	3		3" subdivision	
(516)222-0200	NTD-1	Automatic	-	1.2	1.6	1	±(5 mm + 5 ppm)	1"	$15,985
			-	1.8	2.3	3			
Pentax	PX-10D	Manual	-	1.4	-	1	±(5mm + 5 ppm)	10" digital	$8,450
Englwood, CO			-	1.7	-	3		5" direct	
(303)773-1101								2" estimation	
	PX-06D	Manual	-	1.4	-	1	±(5 mm + 5 ppm)	6" digital	$8,765
			-	1.7	-	3		1" estimation	
Topcon	GTS-2B	Manual	-	1.4	1.7	1	±(5 mm + 5 ppm)	6"	$7,990
Paramus, NJ			-	2.0	2.4	3			
(201)261-9450			-	2.6	3.0	9			
	ET-1	Automatic	-	1.4	1.7	1	±(5 mm + 5 ppm)	2"	$14,250
			-	2.0	2.4	3			
			-	2.6	3.0	9			
Wild Heerbrug	T2000+	Automatic	1.2	2.5	3.5	1	±(5 mm + 5 ppm)	0.5"	$24,000
Farmingdale, NY	DI4L or		1.5	3.5	5.0	3	±(3 mm + 2 ppm)		$25,000
(516(293-7400	DI5		1.7	4.5	6.0	7			
			1.8	5.5	7.0	11			
	T2000+	Automatic	2.0	6.0	9.0	1	±(3mm + 1 ppm)	0.5"	$30,000
	DI20		2.3	7.0	11.0	3			
			2.6	8.0	13.0	7			
			2.7	9.0	14.0	11			

* Atmospheric visibility: a. low = hazy, 5km b. medium = clear, 15km c. high = very clear, 30km

depending on antenna heights and power outputs. Position measurements are indirect (i.e., by timing the travel of multiple pulsed signals from a master to two or more remote stations and back; alternately, phase differences between arriving signals can be measured). Available systems operate in the range-range mode, the hyperbolic mode, or both. The position fix is defined by the intersection point of two position circles or hyperbolic constant-phase lines. Because microwave systems have nominal accuracies of ±1-3 m (±3.3-9.8 ft) from very short ranges to 25-40 km (16-25 mi), they provide adequate positioning capability for survey work and monitoring programs. Potential limiting factors include problems with shore station security, and signal interference in industrial areas or in the vicinity of radar-intensive military installations. Characteristics of representative microwave navigation systems are summarized in Table C-9.

Trisponder--The Del Norte Trisponder is an X-band (8,800-9,500 MHz) positioning system composed of a digital distance-measuring unit (DDMU), a master station (usually on the vessel), and two remote stations located at known geographic positions. Each station is a combined transmitter and receiver. The master station antenna is omnidirectional and each remote station has a directional antenna. Distances to remote stations are observed on the DDMU using the range-range mode. A time-sharing feature allows up to eight users.

The manufacturer quotes a typical range accuracy of ±1 m (±3.3 ft), with an instrument resolution of 0.1 m (0.3 ft). In The Hydrographic Manual, Umbach (1976) cites a range error for this system of ±3 m (±10 ft) with good field conditions based on the Trisponder Basic Operation Manual published in 1974. Also cited were tests conducted by the National Ocean Survey (date unknown), which indicated that temporal electronic drift may cause measurement variations. Recalibration is suggested over a measured base line after every 200 h of operation. However, the tests were conducted on a Model 202 Trisponder Surveyor System for which the manufacturer claimed a resolution of 3 m (10 ft) and a positional accuracy of ±3 m (±10 ft), after field calibration.

The currently available microwave system operates with Model 217/218E transponders and Model 520 or 542 DDMUs. The Model 261 transponder, usable as a master or remote, has a 5-km (3.1-mi) line-of-sight range. The Model 217/218E transponders operate up to 80 km (50 mi) from shore due to higher output power. The transponders are designed for use with either of the DDMU models. The Model 520 will collect four ranges and display two. The Model 542 interrogates four remotes, outputs four sets of data, and also provides a positioning guidance capability. The cost of a complete system, including a Model 520 DDMU, a master and two remote 217E/218E transponders, and antenna, is $40,000 (Buchanan, C., personal communication). For the additional guidance capability of the Model 542 DDMU, the system cost is $44,500. A less expensive "black box" version of the DDMU (Model 562) is available for use with an existing shipboard computer system, allowing more than eight users. Cost of the Model 562 and other required components is $39,500.

TABLE C-9. SHORT-RANGE POSITIONING SYSTEM CHARACTERISTICS

SYSTEM	RANGE (km)	FREQUENCY (MHz)	NOMINAL ABSOLUTE ACCURACY (m)	USER CAPABILITY	ANTENNA Vessel	ANTENNA Shore Station	COST
TRIPSONDER (520/540 DDMU) DEL NORTE Technology, Inc Euless, TX (817)267-3541	5 80 80	9320-9500(261) 9329-9500(217E) 8800-9000(218E)	±1	up to 8 (more optional)	360°H 20°V	87°/180°H 6°/50°V	$40,000
FALCON 484 Mini-Ranger Motorola, Inc. Tempe, AZ (602)897-4376	37	5410-5600	±2	up to 20	360°H 25°V	70°H 15°V	$39,300
MICRO-FIX Racal DECCA Survey, Inc. Camarillo, CA (805)987-8080	80	5480 5520 5560	±1	up to 16	360°H 20°,30°V	90°H 6°/30°V	$43,000
HYDROFLEX Teledist, Inc. Mastic Beach, NY (516)399-5843	100	3000	±1 plus $3x10^{-6}$ distance(m)	single multiuser option	360°H 18°V	Long-range 24°H, 22°V Fan Beam 18°V,60°H	$63,500
AUTOTAPE DM-40A/DM-43 Cubic Western Data San Diego, CA (619)268-3100	150	2900-3100	±0.5 ±1:100,000 range	single	360°H 10°V	30°-120°H 10°V	$90,000

*January 1985

Falcon 484 Mini-Ranger--The Motorola Falcon 484 is a C-band (5,410-5,600 MHz) microwave ranging and positioning system that operates in the two-range mode from 100 m (328 ft) to 40 km (25 mi). The system consists of a vessel receiver-transmitter assembly with an ommidirectional antenna, a range console, and shore-based radar transponders with directional antennae. Pulsed radar from the survey vessel transmitter interrogates radar transponder reference stations located at geographically known points. Elapsed time between transmitted interrogations and the reply from each transponder is used as the basis for range determinations. Two ranges are used for trilateral positioning. If three or four ranges are available, range residuals, sum of squared residuals, and error circle radius data are output for the least squares position solution. The manufacturer claims a range accuracy of ±2 m (±6.6 ft). Up to 20 users can operate in the same area.

The current cost of a basic Falcon 484 system, including a range processor control display, receiver/transmitter with ommidirectional antenna, two reference stations with directional antenna, miscellaneous cables, and manuals is $39,300 (Jolly, J., personal communication). In an effort to eliminate one of the two major problems with most microwave positioning systems (i.e., magneton failures and need to service beacons), Motorola currently is developing a solid-state beacon using a gun diode. The associated increase in long-term reliability also will result in decreased range. However, range capability should remain adequate for positioning needs in Puget Sound. Costs of the modified transmitters were not available at time of publication.

Micro-Fix--The Racal Survey Micro-Fix is a range-range microwave positioning system. With line-of-sight to shore-based transmitters, it is capable of operating up to 80 km (50 mi) offshore. The system normally operates at 5,480 MHz, with options at 5,520 and 5,560 MHz. The master station can interrogate up to eight remote stations (from a possible 32), with each remote transmitter/receiver unit (T/R) preset to recognize its own distinctive station code. Up to four separate station groups can be deployed in the same area without interstation interference. Multiuser capability allows a maximum of 16 users for each deployed chain. Master and remote units are interchangeable.

The basic system consists of a master station with a Control Measurement Unit (CMU), a T/R unit, and two tripod-mounted remote T/R stations. The vessel's master station interrogates the remote stations sequentially, triggering reply pulses received by the master T/R and processed by the CMU to display the corrected ranges. The CMU capabilities include automatic and continuous self-calibration, track guidance, plotter drive, x-y conversion (full spheroid and multi-range solution), and slant range correction. Nominal accuracy is stated by the manufacturer to be ±1 m (±3.3 ft). The cost of a basic Micro-Fix system is $43,000 (Harris, E., personal communication), including training.

The manufacturer is developing the capability to use the system in a hyperbolic mode, a combination range-range/hyperbolic mode, or a range/azimuth mode, but this capability is not yet available. The system also uses circular polarization techniques to avoid reflective (water surface) signal cancellation nulls, thereby eliminating the need for a second antenna on the vessel. This has been accomplished by an antenna design that prevents signal entrance from reflective angles.

Hydroflex--Hydroflex is a short-range to medium-range microwave navigation system designed for survey applications where a high degree of accuracy is required in fixing or tracking a moving vessel. The system operates at frequencies of 2,920 to 3,300 MHz and has a range of 100 m to 100 km (328 ft to 62 mi). It consists of a master unit controlled by an HP85 computer with a customized software package, an ommidirectional antenna, and connecting cables. Each remote unit consists of a transceiver and either a large range or fan-beam antenna for mounting on a customer-provided tripod. Accuracy is claimed to be 1 m (3.3 ft) $\pm 3 \times 10^{-6} D$, where D is the distance in meters. The system can be operated in either a two- or three-range mode. Although single-user is the normal operating mode, a multiuser option is available. The cost of a master and two remote stations is $63,500 (Baker, W., personal communication).

Autotape DM-40A/DM-43--The Cubic Western Autotape is an S-band range-range microwave positioning system that operates at ranges of up to 150 km (93 mi). System components include a shipboard interrogator and range responders at each fixed onshore station. The DM-43 is capable of working with three geographic sites. Range information is computed by comparing the phase shift of the modulated signal transmitted between the interrogator and responder phase unit to an interrogator reference signal. An Automatic Position Computing System (APCS) is available for steering information, real time analog plot of the vessel's track, and magnetic data recording. The system does not accommodate multiple users. The manufacturer claims a range accuracy of 0.5 m plus 1:100,000 times the range distance. This error is due to internal random errors, systematic errors, temperature variation bias errors, signal strength, component aging, and initial calibration errors. External errors are said to far exceed internal noise, systematic, and bias errors. Index of refraction error, which can approach 5 m (16.4 ft) at 100 km (62 mi), is usually small enough at short ranges to be ignored. Multipath rms errors (dependent on orientation of reflective objects near and behind the interrogator omnidirectional antenna) have been observed from 0 to 3 m (0 to 9.8 ft). The manufacturer states that internal averaging plus external data smoothing will reduce the effect on Autotape to a small fraction of a meter, provided the antenna is moving. For this reason, the system is best suited to applications where the interrogator is on a moving vessel.

The cost of a basic Autotape system including an interrogator, two range responders, and associated antennae is $90,000. The same system with the DM-43 and three shore stations is $124,000 (Hempel, C., personal communication).

Medium-Range Systems

Systems in this category typically operate in the medium- to high-frequency bands (i.e., 1.5-400 MHz), achieving greater ranges using EM waves that propagate around the earth's surface. Positional accuracies of medium-range systems vary from a few meters near the base line to tens of meters at the system's range limits (Ingham 1975). Medium-range systems must be used with caution in inland waters due to the severe landmass attenuation and water-land interface effects. Such effects usually are manifested as large calibration variations within a limited survey area. Characteristics of representative medium-range electronic position fixing systems are summarized in Table C-10. Because of the limited availability, high capital costs, and logistical problems posed by the large-sized land stations, these systems are not discussed further herein. Other systems offer similar accuracies at reduced costs and are less time intensive for station setup. Various semi-permanent systems in Table C-10 are discussed in detail in Tetra Tech (1986).

Long-Range Systems

Long-range and global navigation systems generally operate at low (30-300 kHz) or very low (less than 30 kHz) frequencies. As in the case of medium-range systems, EM waves at such frequencies travel for very long distances, typically limited by transmitter power. Onshore station chains usually are permanent, for use with an appropriate vessel receiver and published hyperbolic lattice charts (Ingham 1975). Achievable accuracies typically are much lower than those of shorter-range systems because long-range systems are designed for general navigation rather than accurate positioning. Satellite navigation systems, which operate at much higher frequencies, afford global coverage at much higher accuracies. Characteristics of selected long-range navigation systems are presented in Table C-11.

Loran-C--

Loran, an acronym for long-range navigation, is a pulsed low-frequency electronic navigation system that operates at 90-110 kHz in the hyperbolic mode. Loran-C receivers match cycles to measure time differences between arriving master (M) and secondary (W, X, Y, Z) signals, which are pulse- and phase-coded to enable source identification (Panshin 1979). The usec arrival time differences are displayed and can be plotted on a special Loran-C latticed chart as lines-of-position. Fully automatic Loran-C receivers simultaneously process signals from two master-secondary station pairs, displaying LOP information for tracking.

Range capability varies because Loran-C stations radiate peak powers of 250 kW-2 MW. Due to the use of low frequencies and large baseline distances [i.e., 1,850 km (1,150 mi) or more], Loran-C can provide positional information of reasonable accuracy out to 2,225 km (1,380 mi) with sky waves (Maloney

TABLE C-10. MEDIUM-RANGE POSITIONING SYSTEM CHARACTERISTICS

SYSTEMS	RANGE (km)	FREQUENCY (MHz)	NOMINAL ABSOLUTE ACCURACY	USER CAPABILITY	ANTENNA VESSEL	ANTENNA SHORE	COST*
SYLEDIS Sercel, Inc. Houston, TX (713)492-6688	300	420-450 406-434	±1 LOS ±3 2X LOS	Up to 4 (Range-range) Unlimited (Hyperbolic)	Not Provided		$ 51,350
RAYDIST Hastings-Raydist Hampton, VA (804)723-6531	278 night 740 day	1.5-2.5	±3	4/net (Range-range) Unlimited (Hyperbolic)	Omnidirectional		$102,200
HYPERFIX Racal DECCA survey Camarillo, CA (805)987-8080	700 day 250 night	1.6-3.4	5-10 0.01 lane display resolution	(Range-range) Unlimited (Hyperbolic)	Omnidirectional		$113,000
ARGO DM-54 Cubic Western Data San Diego, CA (619)268-3100	740 day 370 night	1.6-2.0	4-5 0.05 lane display resolution	Up to 12 (Range-range) Unlimited (Hyperbolic)	Omnidirectional		$188,000
HYDROTRAC Odom Offshore Surveys Baton Rouge, LA (504)769-3051	460 day 230 night	1.6-4.0	2-40 0.01 lane display resolution	Single (Range-range) Unlimited (Hyperbolic)	Omnidirectional		$100,000

*January 1985

TABLE C-11. LONG-RANGE POSITIONING SYSTEM CHARACTERISTICS

SYSTEM	RANGE (km)	FREQUENCY (KHz)	NOMINAL ABSOLUTE ACCURACY (m)	USER CAPABILITY	COST
LORAN-C Multiple Receiver Manufacturers	2500 day 1850 Night	90-110	185-460 15-90 repeatable	Unlimited	$1,000-2,000
LAMBDA DECCA Survey, Ltd. England	400-800	100-200	137 day 730 night	Single 2-range Unlimited-hyperbolic	Call Agent
OMEGA Multiple Receiver Manufacturers	Global	10-14	1800 day 3700 night	Unlimited	$4,000-10,000
VIEWNAV Navigation Sciences Bethesda, MD (301)952-5225	Some Coastal Areas	90-110	±10	Multiple	$40,000 and $2,000 annual fee
TRANSIT Multiple Receiver Manufacturers	Global	150 & 400 MHz	90, 1 pass/1 freq. 37-46, 1 pass/2 freq. 3-5, Multiple pass and 2 freq.	Unlimited	$2,500-10,000 basic $30,000-45,000 elaborate
GEOSTAR Geostar Corporation Princeton, NJ (609)452-1130	U.S. Land & Coastal Areas	5117-7075 1618-2492	1-7	Unlimited	Transceiver $450 and link Rental $10-30/mo. (Estimated)
NAVSTAR/GPS Mulitple Receiver Manufacturers	Global	1575 MHz 1228 MHz	40, C/A, CEP 8-9, P, CEP	P-Military C/A Commercial Foreign	$10,000-140,000 initial $1,000 eventually
SERIES ISTAC, Inc. Pasadena, CA (818)793-6130	Global	1575 MHz 1228 MHz	< 1 m diff. Mode	Unlimited	$287,000
AEROSERVICE Houston, TX (713)784-5800	Global	1575 MHz 1228 MHz	< 1 m (1/2 hour)	Multiple	$235,000

1978). Range achievable at a particular station is dependent upon transmitter power, receiver sensitivity, noise or interference levels, and signal path losses (Canadian Coast Guard 1981).

At best, the absolute accuracy of Loran-C in normal operating mode over short distances using the ground wave varies from 185 to 460 m (0.11 to 0.29 mi), whereas repeatable accuracy varies from 15 to 90 m (49 to 295 ft) depending on the vessel's location within a given coverage area (Dungan 1979; U.S. Coast Guard 1974). Achieving the short-range accuracies cited above requires proper installation, maintenance, and operation of high-quality equipment (Canadian Coast Guard 1981). Higher accuracies can be obtained by operating in a differential mode (i.e., with a supplemental receiver at a surveyed site onshore which transmits corrections or offsets to the survey vessel). Available equipment varies from simple receivers and indicators to fully automated receivers with self-tracking capabilities that can interface with a vessel's computer. The cost of a Loran-C receiver, excluding the antenna, varies from less than $1,000 to $2,500-plus.

Although Loran-C frequently is used in Puget Sound for sampling and monitoring, this application has potential problems. Of particular concern when operating in areas such as Puget Sound are time and spatial variations in the Loran-C signals, and signal interferences that prevent operating in desired survey areas. Inland location [up to 160 km (100 mi)] of Loran-C chains requires overland signal transmission. This results in phase shifts that are difficult to predict. Such shifts can cause an erroneous position location fix. There are also anomalies associated with land-water interfaces and large structures such as bridges and tall buildings. Crossing-angles also can widely vary from one geographic area to another. In some cases, lines of position are almost parallel, making an accurate fix very difficult. Noise and interference (e.g., from engines and other electronic equipment) also can be disruptive, but most Loran-C receivers are equipped with factory-set or tunable notch filters to minimize such problems.

In an effort to improve navigational capability using the Loran-C system, the U.S. Coast Guard completed a one-time survey of the east and west U.S. coasts in which Loran-C positions were compared with those from a calibrated microwave system. Corrections were obtained for the Defense Mapping Agency nautical charts, whose LOPs were based on theoretical transmission over water paths (Ryan, R., personal communication). These corrections do not include seasonal or diurnal signal effects. The land transmission-path effect (known as the additional secondary phase factor) currently is under evaluation. The results of a recently completed multi-year West Coast Stability Study, which extended from San Diego to Vancouver Island, indicate that the repeatability of Loran-C in Puget Sound is significantly better than other parts of the country. The annual variation in signals were on the order of 0.2 usec (Figure C-6). The corresponding 95 percent confidence ellipses and 2 d_{rms} positional repeatabilities at Neah Bay are $\pm$40 m (131 ft). As the System Area Monitor at Whidbey Island is approached, seasonal stabilities should improve, resulting in improved positional repeat-

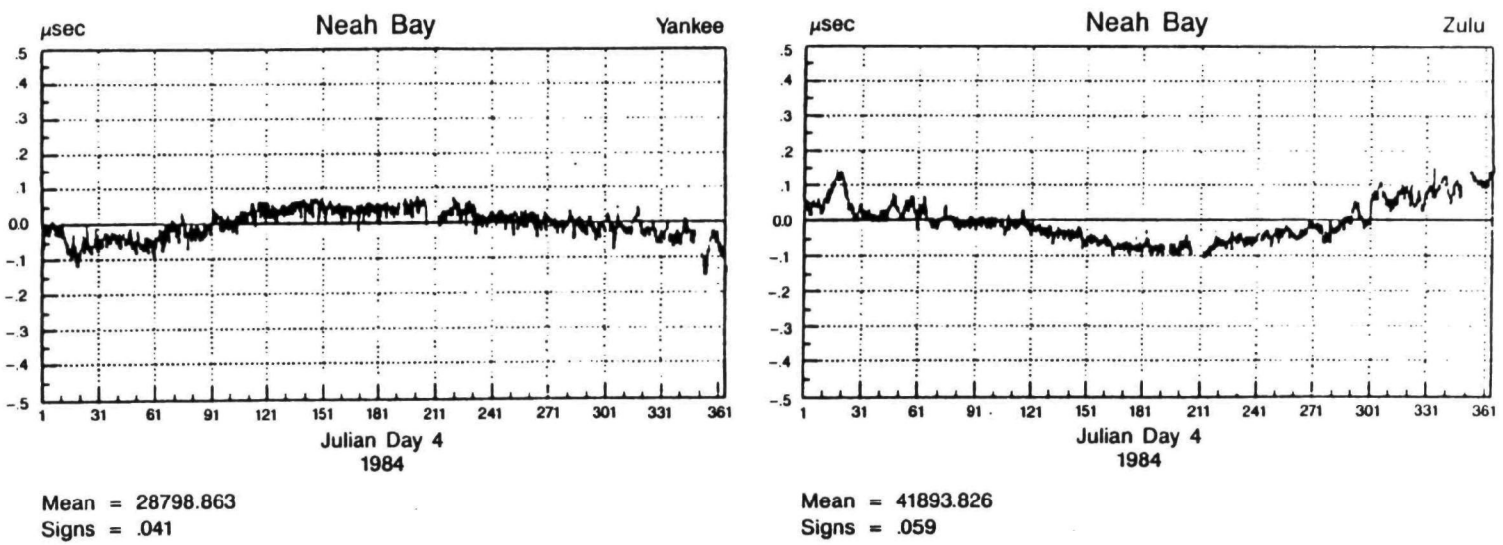


Figure C-6. Seasonal variations in Loran-C signals at Neah Bay, Washington 5990.

ability. Based on the study and the propagation model developed, the Coast Guard can estimate the error ellipse and 2 d_{rms} error circles for most locations within Puget Sound (Slagle, D., personal communication).

Due to differences in the path conditions, measured time differences (i.e., difference in arrival times of two simultaneously transmitted signals) often are different than theoretically predicted. In fact, the deviation varies depending upon the receiver location within the reception area. So-called spatial variation or grid warpage can be removed by applying corrections based on measurements at a nearby site that has been accurately surveyed. Such a procedure was followed by the U.S. Coast Guard during an extensive survey of Puget Sound's major ship traffic lanes. The purpose was to establish accurately located way points to which the vessel can be navigated. Following prescribed turning instructions at a given way point, the vessel proceeds to the next way point, and repeats the procedure until the destination is approached. Given an absolute accuracy requirement of ±40 m (131 ft), the Coast Guard conducted simultaneous measurements at thousands of locations within the Sound with a Motorola Mini Ranger and a Loran-C receiver. As a result, time differences at geodetically known way points have been published and data for many interim track points have been archived. Thus, for these points, a spatial correction can be made. To maximize the absolute and repeatable accuracy at a given way point requires input of an additional correction factor to compensate for the daily and seasonal signal variations earlier addressed.

For purposes of locating sampling stations, it is possible to utilize the way point data to develop a correction for other nearby locations based on interpolation of data from the nearest four way point stations (Gazely, L., personal communication). The accuracy to which the desired time differences can be calculated and therefore the site located is dependent on distance from the way points or track points to the desired location.

Metro completed a similar study with fixes from a microwave system to plot the corresponding Loran-C coordinates within Elliott Bay and surrounding areas. Absolute accuracies within this mapped region are increased to 40-100 m (131-328 ft) with repeatable accuracies substantially better (Sturgill, D., personal communication). Metro and other groups update this Loran-C chart periodically, with a new version expected during the first half of 1986. Because repeatable accuracies of Loran are acceptable for the monitoring objectives, calibration of Loran-C positions is a reasonable alternative for long-term monitoring projects by reducing costs incurred with renting microwave systems (to a single expense). If the study site is within or near the calibrated Loran-C stations set up by Metro or the Coast Guard, then interpolation from the nearest stations may be adequate for accuracy requirements.

Both temporal and spatial variances from a predicted navigation system grid can be substantially reduced or eliminated by operating in a differential mode (U.S. Department of Defense and U.S. Department of Navigation 1984).

A facility (in this case a calibrated Loran-C receiver) may be located at a fixed point within an area of interest. Loran-C signals are observed in real time and compared with signals predicted for the known position. The differences between the observed signal and predicted signal is transmitted to users as a "differential correction" to upgrade the precision and performance of the user's receiver processor. For Loran-C, the serving radius for correction transmission may be up to 320 km (200 mi). The U.S. Coast Guard studied the ability of the differential Loran-C to meet the 8-20 m (26-66 ft) accuracy requirement of U.S. harbors and harbor approaches. For the Seattle area, the Coast Guard Double Range Difference MOD 2 Model predicts that a 20 m (66 ft) 2 d_{rms} absolute position accuracy is feasible when operating in a differential mode (Doughty and May 1985). Tests using Least Squared Error, Alpha-Beta Filter, and Linear Regression Model approaches to predict differential Loran-C time difference offsets indicate that a consistent accuracy within 10 m (33 ft) is not an unreasonable goal for the particular differential Loran-C system examined on the Thames River (Bruckner 1985).

Radio frequency (RF) interference was evident during the West Coast study, both nearby and within the Loran-C band of 90-110 kHz (Blizard and Slagle 1985). RF interference was noticeably observed at the Tacoma Washington Harbor Monitor Site, and directly affected the Coast Guard's data acquisition and collection. During the 16 months of operation at this site, a significant amount of the data was considered of poor quality, attributable to the intermittent U.S. Navy transmissions at 76.3 kHz. Apparently the strength of the signal is so strong that it caused the 100 kHz tuned Loran-C coupler to oscillate. Conversations with area users indicated that many other types of receivers also experienced similar problems, thereby limiting the use of Loran-C in the Tacoma area. The extent to which the problem is experienced further away from Tacoma will be receiver-dependent. The U.S. Coast Guard found that use of notch filters in the Hood Canal/Bremerton area eliminated the problem. Identifing the limit of the interference area would require transits away from the source area while attempting to notch out the interference, a task not included in the Coast Guard study.

Other areas with signal interference include relatively small intermittent sources in Elliott Bay and at Seola Beach near Sea-Tac Airport (Figure C-7). A large region of northern Puget Sound often is affected apparently because of another U.S. Navy transmitter. This source precludes reception from most sets north of Everett (in an area with normally good Loran reception).

Two additional sources of interference found in the West Coast study were transmitter switching and chain control effects. The AN/FPN-44A tube transmitters in the U.S. West Coast Chain (9940) located at Fallon, Nevada (Master); Middeltown, California (X-Ray); and Searchlight, Nevada (Yankee) are switched every 14 days for routine maintenance, resulting in a signal offset for approximately 28 days. The resultant 40 nanosecond error/offset

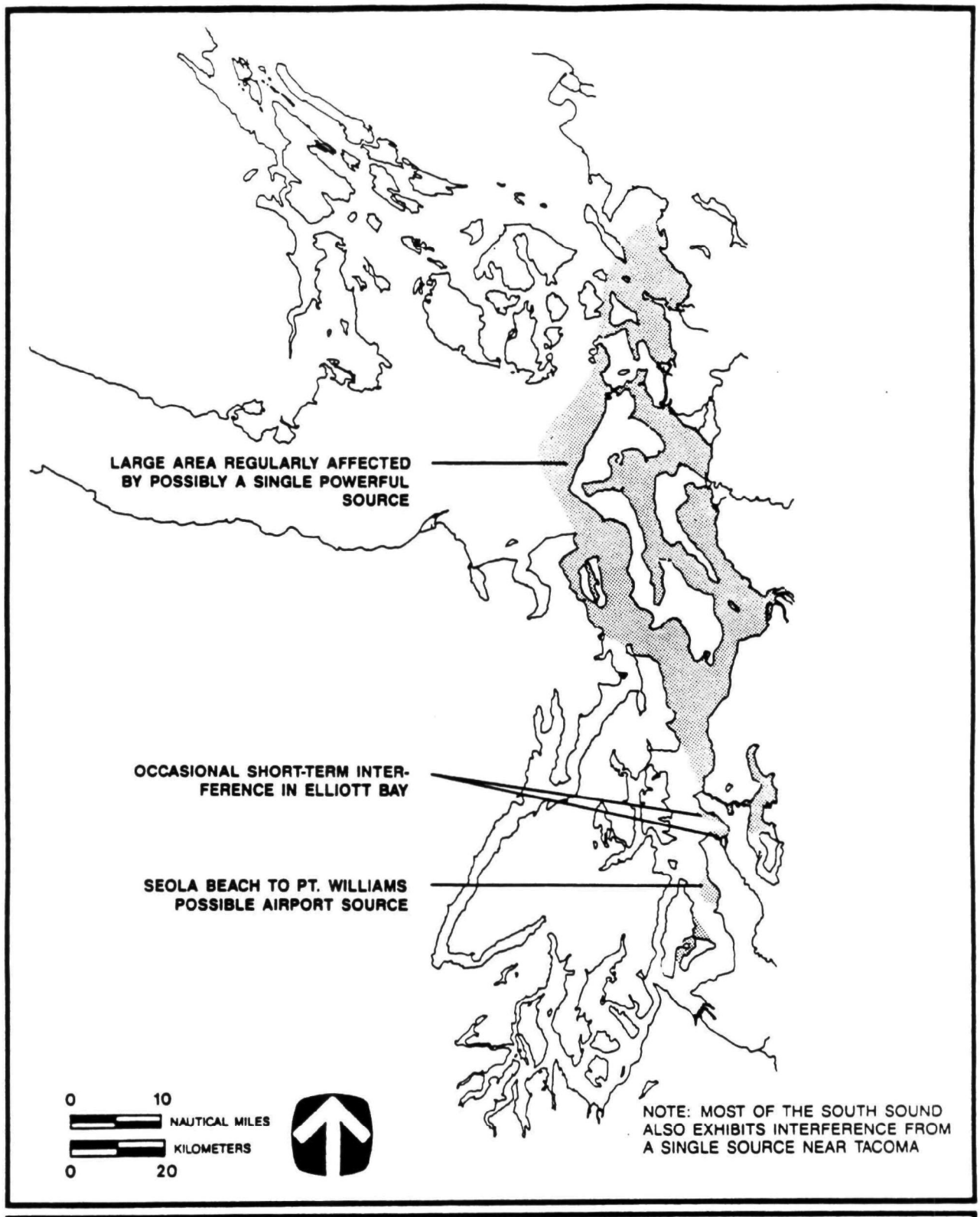


Figure C-7. Regions of LORAN-C signal interference in Puget Sound.

would most likely be detected and applied in the time difference to the latitude/longitude conversion algorithm. The Canadian West Coast Chain (5990) would most likely exhibit similar offsets.

Also apparent in the study was the effect of moving an Alpha-1 site receiver that maintains the Control Standard Time Differences. When the 9940 Whiskey A-1 Monitor was moved in December 1984, a positional grid shift of 300 nanoseconds occurred. For a typical gradient of 305 m (1,000 ft) per usec, this is equivalent to a 91.4 m (300 ft) change in position. Thus, applying Loran-C in a repeatable mode, the user would have found his "past" position moved by more than 91.4 m (300 ft). In fact, depending on crossing angle, the difference in positions could even be more. Thus, users in the repeatable mode must monitor and compensate any changes in the U.S. West Coast or Canadian Chain (whichever is used) when they occur.

In summary, the accuracies of Loran-C vary depending upon the location. Prior knowledge of the area reception is required for an adequate determination of its useability. Achievable accuracies have to be acceptable to the sampling/monitoring objective for the system to be considered. However, because of its relatively low cost, ease of installation/ operation, and reliability in known areas over the range of conditions, Loran-C is a candidate system to be considered.

Viewnav--

To improve upon the positioning accuracy of standard Loran-C receivers, Navigation Sciences has developed Viewnav, an interactive computer system that uses differential Loran-C to position a vessel with a claimed repeatable accuracy of 4.6 m (15 ft). Absolute accuracy of the system is on the order of ±10 m (±32.8 ft) at the 90 percent confidence level, and ±5 m (±16.4 ft) at the 20 percent confidence level (Newcomber, K., personal communication). Loran-C offsets are obtained by interrogating onshore monitors established by the company. In addition, a land-based microwave system is used to calibrate a vessel's initial position or track. The system is particularly effective in ports and harbor areas where large buildings or the land-water interface may alter Loran-C readings.

A distinctive feature of the system is an electronic display of the survey area based on digitized nautical charts. As the vessel moves, its display position moves relative to depth contours and land boundaries. Other waterborne radar images in the area also are indicated.

A full system costs approximately $40,000 with a supplementary annual service fee of $2,000 for chart corrections and equipment maintenance. The base price includes a mainframe Ai-M16 computer with 512KB of main memory, a flexible disk drive with 1MB capacity, and a Winchester hard disk of 10MB capacity. The basic system provides 5MB of chart storage,

which equates to approximately 650 charts depending on scale selected. Additional charts can be stored on floppy disks. The manufacturer expects charts and Loran monitors throughout U.S. coastal areas by 1986.

The system could provide improved accuracies over Loran-C, especially in areas of Puget Sound that have not been mapped for Loran. The shore stations require setup time, as does the initial position calibration, making this system more attractive to long-term monitoring programs. Loran-C reception limitations again cause limited coverage of the Sound.

Lambda and OMEGA--

These two long-range systems are offshore positioning systems that do not provide adequate accuracies and/or coverage for station positioning within Puget Sound. Interested parties are referred to Tetra Tech (1986) for more information on these systems.

Transit (Navsat)--

The U.S. Navy Navigation Satellite System (originally Project Transit) consists of a group of satellites in 106-min circular polar orbits at altitudes of approximately 1,411 km (877 mi). The system also includes ground tracking stations, a computing center, an injection station, U.S. Naval Observatory time signals, and vessel receivers and computers. Positional measurements are based on the Doppler frequency shift that occurs when the relative distance between the satellite transmitter and vessel receiver changes (i.e., frequency increase upon closure and frequency decrease upon separation). Provided the satellite orbits are accurately known, it is possible to locate the receiver. The nature of the Doppler shift depends upon the exact location of the receiver relative to the satellite path (Maloney 1978). The system operates at frequencies of 150 and 400 MHz so that ionspheric corrections can be made through signal comparison techniques. The vessel's position is determined based on "known" orbital positions during satellite passage and measured frequency shifts.

As originally designed, at least one satellite would be within line-of-site every 35 to 100 min. However, at U.S. East and West Coast latitudes, the acceptable fix window is approximately every 90 min (Driscoll, C., personal communication). This is caused, in part, by the requirement that a satellite's maximum altitude be between 15° and 75° before a fix is considered valid. Another problem occurs when two satellites being tracked have approximately the same closest approach, whereupon it becomes difficult to know which one is monitored. Typically, each satellite provides four fixes a day on two successive orbits spaced by 12 h. Because one satellite currently is inoperative and another has weak batteries, it may take longer (e.g., several hours at the equator) to gain a valid fix (Booda 1984).

A static position fix with Transit using single-channel equipment can be made with an accuracy of approximately 90 m (295 ft). Dual-channel

receivers improve single-pass accuracy to 37-46 m (121-151 ft) (Hoeber 1981; Maloney 1978). With multiple passes, an rms accuracy of 3-5 m (9.8-16.4 ft) is claimed by some equipment manufacturers. Using translocation (i.e., simultaneous measurements at two fixed locations, one of which is accurately known), estimated positional measurement accuracies are in the range of 0.5-1.5 m (1.6-4.9 ft) (Driscoll, C., personal communication; Moyer, C., personal communication). Magnavox has developed a satellite surveying program (MAGNET) that is reportedly capable of simultaneous location of up to 10 sites with a point-to-point accuracy of 30 cm (1 ft) or better. However, this system is designed for coordinated geodetic studies over wide areas using 3 to 10 MX 1505 receivers and recording data from 10 or more satellite passes (Anonymous 1981).

Transit receiver costs range from $2,500 to $10,000 for basic single frequency units (Murphy, W., personal communication). More elaborate multiple-channel systems, sometimes in combination with OMEGA, range in cost from $30,000 to $52,000 (Jolly, J., personal communication; Driscoll, C., personal communication).

Use of the Transit system generally is not appropriate for sampling and monitoring programs because of the inaccuracy of the system for fixes from either a moving vessel or one occupying stations for a relatively short time. A fix must be based on a single pass. With satellite passes at 1-h or 2-h intervals, multiple-pass data acquisition is impractical. Therefore, only the best single-pass accuracy of 37-46 m (121-151 ft) can be achieved. Since translocation is designed for two fixed stations rather than one on a moving sampling vessel, and since MAGNET applies to land surveying only, neither method will provide a significant increase in accuracy during the limited time the vessel will be at any one station. However, the largest disadvantage of the system still is the time constraint between fixes, which does not make it appropriate for surveys using expensive ship time.

GEOSTAR--

GEOSTAR is a pulse radio transmission system. Just approved by the FCC, it will provide satellite information for positions within the continental U.S. and its coastal waters by 1987. Three geosynchronous satellites (and a fourth as backup) will orbit the earth at 37,000 km (22,991 mi) at 70°, 100°, and 130° W longitude. System components include transceivers, satellites, and computers at a ground center. The links between the ground station and the satellites will operate at 5,117-5,183 MHz and 6,533 MHz, while user-satellite links will be at 1,618 and 2,492 MHz (Whalen 1984). Should a satellite fail, the backup would be moved into a proper orbit by telemetry command from the ground computer facility.

The user will send a command through the transceiver, which relays the message through the satellites to a central computer at the ground center, reportedly in less than 1 sec. The signal-arrival times from each

satellite are used by the ground computer to calculate the position of the specially coded transceiver. The information is then transmitted back to the satellites and relayed back to the transceiver in a similar amount of time.

GEOSTAR will enable a typical single-shot positioning error of ±2-7 m (6-23 ft), according to the developer. When needed, accuracies down to ±1 m (3.3 ft) reportedly can be achieved with two-way interaction, signal analysis, and averaging. Users at a known elevation (e.g., sea level) will have greater accuracy due to much smaller geometrical dilution of precision where only two (rather than three) coordinates are required. Continuous operations in a differential mode also should enable correction inputs for such errors as ionospheric delays, satellite position drift, and drifts in satellite electronic delays.

System designers estimate that when operable the cost of a basic hand-held transceiver with a typewriter keyboard and LCD display will be less than $1,000. A monthly service charge in the range of $10 to $30 also is anticipated (Howarth, C., personal communication). At publication, FCC had just approved GEOSTAR Corporation's application for use of the requested frequencies. Candidate users are urged to confirm FCC approval, verify the latest satellite/ground station operating schedule, and obtain further information on transceiver availability.

Navstar GPS--

The Navstar Global Positioning System (GPS) is a second-generation satellite navigation system currently under development by the U.S. Department of Defense. Its purpose is to provide precise, continuous, worldwide, all-weather, three-dimensional navigation for land, sea, and air applications. Under current plans, 18 satellites will be launched into three co-planar orbits 120° apart to provide continuous transmission of time, three-dimensional position, and velocity messages to system users. The GPS satellites transmit at 1,227.6 MHz and 1,575.4 MHz to permit the measurement and correction of ionospheric refraction error. Five developmental satellites currently are in orbit, providing approximately 4 h of coverage twice daily, separated by a 12-h period. Continuous two-dimensional positioning information should be available with 12 satellites by February, 1988, and three-dimensional coverage is projected for late 1988 or early 1989 (DeGroot, L., personal communication; Stansell 1984). The system consists of the satellites in 12-h, 20,200-km (12,552-mi) orbits, a U.S. master control station, several monitoring stations, and small, lightweight, relatively inexpensive receivers. Signals received from any four Navstar satellites are demodulated, time-correlated, and processed to obtain precise position information.

Two levels of positioning accuracy are achievable with the Navstar GPS system. The lower level is obtained from the Standard Position Service (SPS) using the coarse acquisition or "C/A code." When the system becomes fully operational, navigational accuracy from these signals should be approxi-

mately 100 m (328 ft) two-dimensional rms, or a circular probable error (CEP) of 40 m (131 ft) (Montgomery, B., personal communication). More accuracy can be achieved using the Precise Positioning Service (PPS) or "P-code" (i.e., 8-9 m two-dimensional CEP). Additional positioning accuracy can be achieved by operating in a differential mode, in which receivers on a vessel and at a known onshore location simultaneously receive the satellite signals. The onshore receiver is calibrated. Bias corrections based on signals received at the fixed station are transmitted to the mobile receiver. These area-specific corrections yield more accurate positional determinations. With differential GPS, a two-dimensional position should be definable within a range of 2-5 m (6.6-16.4 ft) (Montgomery 1984; Stansell 1984).

Due to the present lack of full-time coverage, both SPS and PPS are available to military and civilian users. However, the government intends to encrypt the P-codes, allowing use only by the military and other authorized users [e.g., National Ocean Industries Association (NOIA) members]. NOIA has proposed that all P-code receivers be owned and operated by a single service company that would provide both the equipment and personnel when national security conditions are met and no other reasonable navigational alternatives are available (Stansell 1984). Under U.S. Department of Defense policy, access to PPS is available to civilian users operating in the "national interest" (Anonymous 1985). Equipment, cryptography, and support services will be provided under contract to the user. However, it would appear that general users in coastal areas will be limited to C/A code equipment. It may therefore be necessary to operate in the differential mode in order to achieve the positional accuracies needed for survey and monitoring requirements.

Because the GPS system is in a developmental stage, cost estimates for the equipment are difficult to make. Several major equipment manufacturers are in the process of designing receivers with varying capabilities, and a limited number of models now are available. Some manufacturers envision that receivers with 100-m (328-ft) accuracy will cost less than $500 when mass produced (e.g., for automobiles). At the other extreme, for $140,000 Texas Instruments sells the TI4100 Navstar Navigator, said to be capable of slow dynamic positioning within a few meters, speed within tenths of a knot, and time to the usec (Montgomery 1984; St. Pierre, R., personal communication). Motorola anticipates that the initial cost of two stations needed to operate in the differential mode will be in the $100,000-range (Sheard, S., personal communication). Magnavox has a five-channel T-Set GPS Navigator, with real time differential GPS operation as a planned option. A two-unit system, excluding communications link, costs approximately $100,000 (Driscoll, C., personal communication). Rockwell International sells a prototype C/A code receiver for $17,500 and anticipates that GPS receivers will cost less than $10,000 by 1988 (DeGroot, L., personal communication). Tracor expects initial models to sell for less than $10,000, falling to around $1,000 in 3-5 yr (Murphy, W., personal communication).

Using the C/A code in a differential mode, the Navstar GPS positioning system should provide accuracies within ±3 m (±9.8 ft). Errors of 1 m (3 ft) at a range of 200 km (12.4 mi) from the reference station are predicted for daytime operation. Smaller errors are expected at night (Kalafus 1985). However, testing with the actual satellite signals modified to reduce achievable accuracy will be required to confirm this capability. The two required stations may be expensive at first, but competition and the large potential market are expected to drive the price much lower.

SERIES--

The SERIES positioning and navigation system (Satellite Emission Range Inferred Earth Surveying) has evolved from a technique originally developed to measure movements in the earth's crust for tectonic studies and earthquake predictions. The system enables use of signals from existing Navstar GPS satellites with no knowledge of either the P or C/A codes by simultaneous pseudoranging to multiple satellites in a differential mode. Accuracies on the order of 2 m (6.6 ft) CEP or 5 m (16 ft) two-dimensional rms are said to be achievable for vessels in a slow dynamic mode (0-10 kn) (ISTAC 1984). This is accomplished by comparing Navstar signal arrivals at one receiver to the same signals received at another shore-based geodetic reference mark. By explicit differencing of the frequency and phase measurements observed from each satellite, it is apparently possible to eliminate the effects of Navstar signal variations that secure the satellites from an unauthorized user in an autonomous, single-receiver, real time mode. SERIES receivers are codeless spectral compressors that simultaneously receive and incorporate spread-spectra modulations of several Navstar satellites and then extract the frequency and phase of each satellite in view. Data are processed by a combination of Doppler positioning and phase ranging.

System components include an unattended, fixed reference station, a marine vessel station, and a reference link (via satellite or terrestrial radio) to transfer the reference station signal to the marine vessel for real time positioning. The reference station includes an SERIES GPS receiver (MPS-1), a rubidium frequency reference, and a customer-supplied radio transmitter (200 Hz analog signal). The marine station consists of the GPS receiver with an integrated Navstar C/A-code-correlating subsystem for broadcast satellite ephemerides, a rubidium frequency reference, a microprocessor for real time position computations, and a customer-supplied receiver for fixed reference station signal reception. A standardized RS-432 interface is provided for communication of the position information to the vessel's navigation system.

The initial MPS-1 units are estimated to cost $205,000 for a ship system and $82,000 for the fixed base system required for differential mode operation (Whitcomb, J., personal communication). The system will probably be available on a lease or service-contract basis, as well.

When operational, the SERIES system may offer a high-accuracy alternative to the Precise Positioning Service of Navstar GPS or to differential mode operation with the C/A code. Although the projected cost for an initial unit is high (to recover research and development expenditures), cost should decrease with further production. Potential users should confirm price, verify accuracies achievable from a mobile marine vessel, and identify specific data-link components that need to be supplied by the user.

Aero Service GPS--

The Aero Service Division of Western Geophysical Company of America is developing a codeless satellite navigation system for marine positioning. The system will enable real time navigation in the slow dynamic mode to less than 5 m (16.4 ft). For vessels in the semi-static mode (on station) for 30 min, the system will reportedly yield a position fix accuracy of less than 1 m (3.3 ft) (Mateker, E., personal communication). The system will require one unit onshore at a fixed position, and one unit on the survey vessel. Continuous real time communication between the two systems may or may not be necessary, depending on steps by the U.S. Department of Defense to degrade certain elements of the GPS satellite signals. Prototype testing was scheduled for September, 1985, with production systems planned for early 1986.

This future system is worthy of note because it will probably be based on methods now used for land surveying in the MACROMETER Interferometric Survey System. The MACROMETER is designed for very precise positioning in a fixed-point (static) mode, using GPS signals but no codes. Company tracking stations (Phoenix, Arizona and Woburn, Massachusetts) will follow up to six satellites simultaneously. The MACROMETER is capable of providing position in terms of latitude, longitude, and ellipsoidal height to within a few parts per million of the distance from a reference point. In the continental U.S., 1-3 h of observation will yield first-order (1:100,000) accuracy in all three coordinates using the GPS satellites. Data for a second-order or third-order survey point may be collected in less than 1 h (e.g., as little as 15 min of observation).

The MACROMETER surveying system includes two or more field units and an office data processer. Each V-1000 field unit includes an antenna, receiver, tape drive, 30 m of cable, time receiver, power package, battery chargers, generator, and DC/AC inverter. The P-1000 Office Data Processer computer subsystem includes hard and floppy disk drives, video terminal, tape drive, printer, modem, and all necessary software. A 1,000 series two-unit surveying package is currently priced at $235,000. The system can be rented for $5,000 per V-1000 unit per month, with a 2-mo minimum on each unit.

RANGE-AZIMUTH SYSTEMS

A number of hybrid positioning systems combine positional data from various sources to obtain fixes. Such systems usually involve the intersection of a visual line-of-position with an electronic line-of-position (Umbach 1976). Visual data may be in the form of sextant angles or theodolite azimuths. Electronic positional data are normally obtained from a microwave system.

Of particular interest for survey and monitoring programs are dynamic positioning systems that require only a single shore station and that use the simultaneous measurement of angle from a known direction and range to the survey vessel. This range-azimuth method has the advantage of circular coverage around the shore station (Figure C-8). A single station minimizes logistical requirements and geometric limitations. Line-of-position intersections are the ideal 90° everywhere within the coverage area. Growth in the error ellipse is due only to distance from the shore station because of its independence of the absolute azimuth angle. Accuracy improves as range decreases, even fairly close to the shore station, and only one unobstructed line-of-sight is needed. However, such systems allow only a single user.

Characteristics of three representative systems are summarized in Table C-12. Two have fully automatic shore stations, requiring attendance only during setup and alignment. The third requires an onshore operator at all times. For multiple-day surveys, an automatic station eliminates the tedium of continuously tracking the survey vessel. The systems offer much greater flexibility than range-range or hyperbolic systems with multiple onshore stations. Where positioning requirements extend into ports, estuaries, or up rivers, the single-station systems offer distinct advantages in covering restricted or congested survey areas and in establishing an unobstructed signal path. Each system is distinctive in either its operating medium (optical, microwave, laser) and/or procedure (i.e., manual or automatic tracking). The added costs of these systems over standard microwave systems may be justified where the system also can be used for onshore work during nonsurvey periods.

AZTRAC

The ODOM Offshore Survey AZTRAC is a semiautomated optical angle-measuring and transmitting system which can be used in conjunction with an independent distance-measuring system to position a vessel. The AZTRAC system consists of a modified Wild T16 theodolite, an onshore transmitter, and a vessel receiver. The theodolite has an infinite tangent drive and provides information in a digital format. Typically, the separate distance-measuring system consists of a microwave master receiver on the vessel and a remote unit transmitter located at the theodolite.

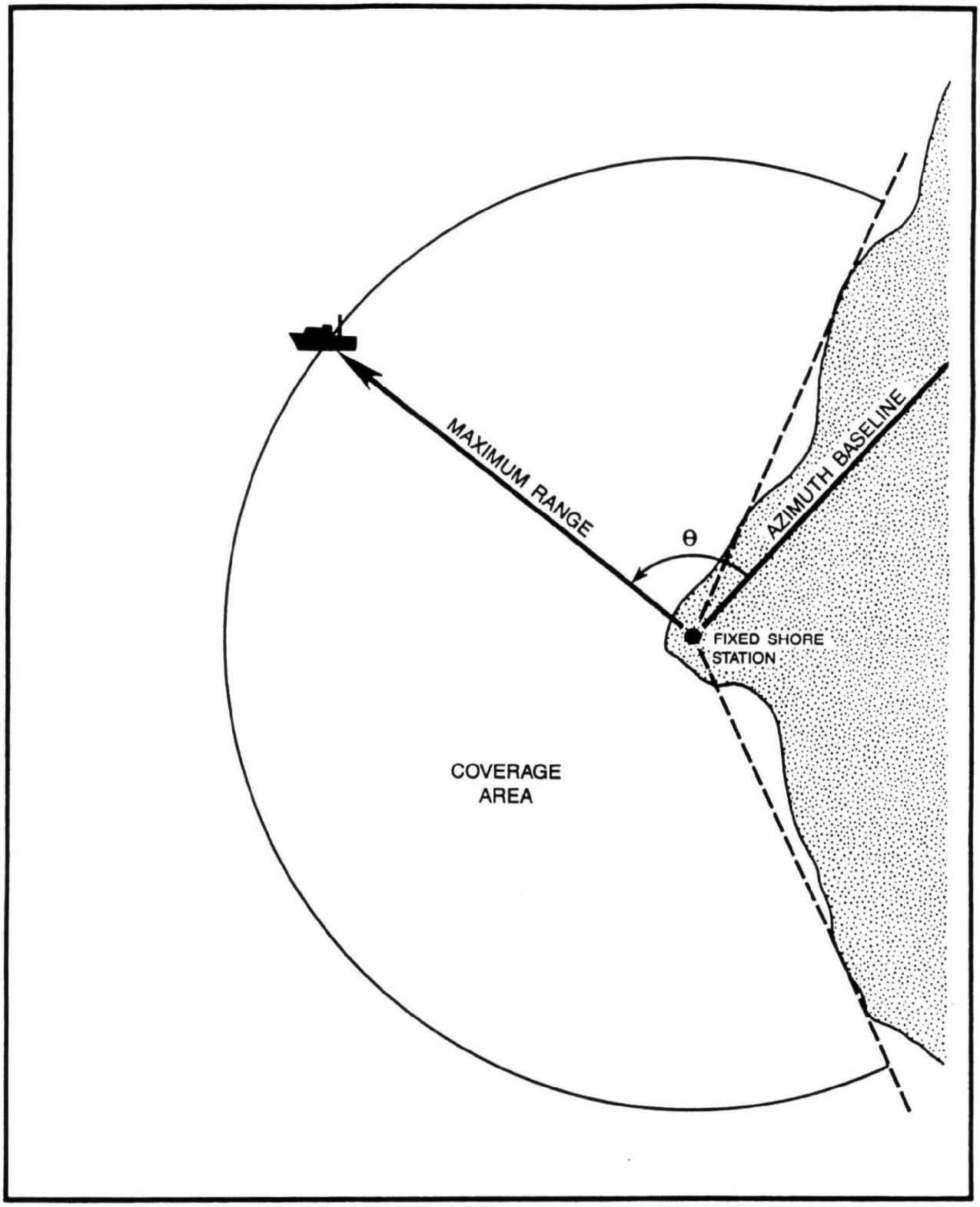


Figure C-8. Range-azimuth positioning system area of coverage.

TABLE C-12. RANGE-AZIMUTH POSITIONING SYSTEM CHARACTERISTICS

SYSTEMS	FREQUENCY	RANGE	NOMINAL ACCURACY AZIMUTH	RANGE	POSITION	COST
AZTRAC Odom Offshore Surveys Baton Rouge, Louisiana (504)769-3051	Optical and microwave (typical)	Visible	0.01°	Both depend on accuracy of ranging system used		$22,500 plus ranging system cost cost
POLARFIX Krupp Atlas-Elektronik Webster, Texas (713)338-6631	Laser (904 nm) UHF Telemetry (406-470 MHz)	5000 m	0.01°	±0.1m±0.1m/km	±0.1m±0.2m/km	$100,000
ARTEMIS Andrews Hydrographics Houston, Texas (713)558-2236	9.2-9.3 GHz	10-1400 m or 200-30,000 m	0.03°	0.5 m short 1.5 m long	Not stated	$70,000 to $75,000

For a survey, the AZTRAC theodolite and transmitter are set up at a known position and the theodolite is zeroed on a known azimuth line of backsight. The theodolite operator sights and tracks the survey vessel's ranging antenna or transponder. As the vessel moves, the microwave ranging system continuously measures distance between the shore station and the vessel. The tracking motion produces pulses that are decoded by the AZTRAC transmitter and displayed to the theodolite operator as the angle to the vessel from the reference azimuth line. The angle also is converted to BCD serial format and is used to activate the transmitter, which sends the information to the survey vessel. The AZTRAC receiver converts the angle information to parallel format and displays it for manual recording. It simultaneously outputs the angle as serial data for automated recording or processing by any available on-board computer or plotting system.

AZTRAC is designed to operate with most microwave ranging systems. Two AZTRAC units working in an azimuth-azimuth mode can provide positioning in survey areas where reflections from metal structures or electrical noise from radar and other transmitters limit use of microwave ranging. The Wild T16 theodolite has a 30X magnification, 27-m (89-ft) field of view at 1,000 m (3,280 ft), and an angular resolution of 0.01^{o} (36 arc sec); at a distance of 5 km (3.1 mi), this corresponds to an absolute arc length error of 0.9 m (3.0 ft).

The National Ocean Survey recently examined range-azimuth positioning of a vessel moving at a nominal speed of 6 kn (11.1 km/h) at ranges of up to 3,000 m (9,842 ft). Instruments included a Wild T2 theodolite, from which angles were manually recorded onshore, and the AZTRAC, whose angles were recorded on the vessel. Pointing errors (68 percent probability) of these two instruments were found to be approximately 1.3 m (4.3 ft), independent of range when standard deviations of right and left movement data were pooled (Waltz 1984).

The theodolite was sited on a white Del Norte Trisponder transponder on the moving vessel. Visibility was good during the 2-day survey (off Monterey, CA), with calm mornings giving way to afternoon winds of 15 kn and 0.6-0.9 m (2-3 ft) seas. Range could have been extended much farther under such conditions, particularly if color had been added to the vessel target (Waltz, D.A., personal communication). U.S. Army Corps of Engineer users confirmed the effectiveness and reliability of the AZTRAC system in conjunction with several different microwave ranging systems (Ard, R., personal communication). The system also has proven effective in port and harbor surveys when it was impossible to achieve optimal shore station geometry for range-range or hyperbolic operation.

The current cost of the AZTRAC alone is $22,500 (Apsey, B., personal communication). A system consisting of the AZTRAC, a Motorola Falcon 484 with one reference station (approximately $32,000), and an interface unit ($10,000) would cost $64,500. Thus, provided the required range is achievable

under anticipated visibility conditions, the additional versatility of the AZTRAC can be realized for $25,000 above the $39,300 cost of a two-station Falcon 484 range-range microwave system.

POLARFIX

POLARFIX is a dynamic range-azimuth positioning system by Krupp Atlas-Elektronik. The system uses a scanning (30° horizontal) pulsed laser beam from a single, fixed, onshore tracking station to follow the survey vessel (up to 10°/sec) and to transmit range and angle information via telemetry link. The system incorporates a fully automated onshore tracking station, which requires no attendance beyond initial station setup and azimuth referencing. The shore station can locate the mast-mounted prism reflectors, follow the vessel, and, if necessary, relocate the vessel by performing a routine search pattern based on a record of tracking history. The shore tracking station consists of a laser-sensing head mounted on a conventional survey tripod, linked by cable to an integrated control unit that houses data control, transmission, and telemetry transceiver equipment. An integral control unit (including a display and a second telemetry receiver), keyboard terminal, printer, telemetry antenna, and prism reflector assembly are onboard the vessel.

Under clear operating conditions, a 3-km (1.9-mi) range using a Class I laser or a 5-km (3.1-mi) range using a Class IIIa laser may be selected. In foggy weather, range is said to be 1.5 times visible range, due to use of the pulsed infrared laser. Maximum range achievable varies with the prism assembly used to reflect the tracking station's laser beam. Single-, dual-, and triple-ring omnidirectional prism assemblies can be stacked on the vessel antenna. For average weather conditions, a two-prism assembly (each with five reflectors) gives approximately 3.5 km (2.2 mi) of range. This can be extended to approximately 5.0 km (3.1 mi) with the addition of more assemblies. Although a 5 km (3.1 mi) distance from shore will cover most of the Sound, this does add limitations to range in some areas.

Range accuracy is reported as 0.1 m ±0.1 m/km (0.3 ft ±0.5 ft/mi) of measured range. Azimuth accuracy is said to be 0.01° or better. The resulting positional accuracy at 1, 3, and 5 km (0.6, 1.9, and 3.1 mi) is approximately 0.3, 0.6, and 1.0 m (1, 2, 3.2 ft), respectively. The positional algorithm given is ±0.1 m ±0.2 m/km (±0.3 ft ±1.1 ft/mi). Current cost of the system is $100,000 (Guillory, J., personal communication).

ARTEMIS

The ARTEMIS by the Christiaan Huygenslabortorium (Holland) is a distance-bearing type of microwave positioning system capable of measurements at ranges of 10 m (32.8 ft) to 30 km (18.6 mi), and angles from 0° to 360° from a single fixed shore station. Accuracies at the two-sigma or 95 percent level are given as ±1.5 m (4.9 ft) distance, and ±0.03° azimuth, equivalent to ±0.5 m/km (2.6 ft/mi).

Angle measurements are based on automatic tracking antennas on the vessel and at the shore station. Once locked, the two antennae move always pointing towards each other. A maximum combined tracking speed of 3°/sec is allowable to achieve the specified angle error. The direction of the fixed station antenna is accurately measured with a precision shaft coder, which is mechanically coupled to the main shaft of the antenna. Measured angle data are transferred to the mobile station via the established continuous microwave channel. The same microwave link is used to measure distance by controlled interruption of the microwave signal. Both angle and distance usually are displayed on the Mobile Control Data Unit, although readout at the shore station also is feasible. The microwave link also is used for voice communication between the two stations without disturbing the data being transmitted.

The vessel's positioning equipment consists of the Mobile Control Data Unit (MCDU), a Mobile Antenna Unit (MAU), the antenna, cables, a telephone handset, and a speaker. The shore station consists of a Fix Antenna Unit (FAU), Fix Control Data Unit (FCDU), an antenna, cables, a telephone handset, a speaker, and a telescope for initial directional alignment. The shore station requires attendance only for setup, referencing, and periodic battery checks. The bearing is electronically referenced to a geodetic grid by siting the unit in a known reference direction and manually adjusting the observed direction readout to the correct value.

Limiting factors (common to all microwave systems) include radio line-of-site conditions (obstacle-free for optimum performance) and multipath interference due to reflections from the sea surface. The latter can be reduced by proper adjustment of antenna heights. Where survey area traffic is heavy, signal interruptions could unlock the two tracking antennae. Manual relocking by a shore station attendant or automatic relocking using autosearch, an option available at added cost, would then be necessary. Although signals generally are unaffected by rain and fog, there is some range reduction during heavy rains or snowfalls.

The present system costs $70,000-$75,000, depending on the options selected (Coupe, C., personal communication). The system may be rented from Andrews Hydrographics, Inc. (Houston, Texas) for approximately $850 per day.

APPENDIX C

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APPENDIX D

EVALUATION OF POSITIONING METHODS

APPENDIX D

EVALUATION OF POSITIONING METHODS

Methods to position at stations in Puget Sound for collection of environmental samples are addressed in this appendix. After an overview of each method is presented, the available positioning methods are evaluated and comparative information is presented to facilitate selection of a method appropriate to meet sampling objectives. Positioning methods and associated equipment are described in detail in Appendix C.

POSITIONING PROCEDURES IN USE IN PUGET SOUND

Equipment, procedures, advantages, and limitations of positioning methods used in Puget Sound were identified from interviews with various governmental and private groups that conduct environmental sampling within Puget Sound (Table D-1). These methods, their accuracies as expressed by the field personnel who work with them, and the limiting restrictions are summarized in Table D-2. The majority of the sampling done in Puget Sound in recent years has been accomplished with the following five positioning methods:

- Sextant angle resection
- Variable range radar (VRR)
- Loran-C
- Combinations of VRR, visual fixes, and Loran-C
- Microwave systems.

Many smaller research vessels in Puget Sound are equipped with VRR and positioning by this method is usually used when VRR fixes are considered adequate for station location definition. However, if higher accuracies are required, VRR fixes are supplemented by visual line-of-site fixes, bottom depth readout, and Loran-C. Loran-C cannot initially be used to locate a station in most areas of Puget Sound. Loran-C coordinates are recorded, however, to help improve repositioning accuracy. Overall, the combination of methods should increase accuracies closer to shore (visual fixes) and with steeper bottom slopes (fathometer).

Of the other methods practiced in Puget Sound, sextant angles are used the least. Because of visibility effects, it is difficult to estimate the accuracy of this method, which can change considerably between stations.

TABLE D-1. INTERVIEWED GOVERNMENTAL AND PRIVATE GROUPS THAT CONDUCT SAMPLING IN PUGET SOUND

GOVERNMENTAL AGENCIES AND INSTITUTIONS

Federal

National Marine Fisheries Service (NOAA)
National Ocean Survey (NOAA)
Pacific Marine Environmental Laboratory (NOAA)
U.S. Army Corps of Engineers
U.S. Environmental Protection Agency

State

Department of Ecology
Department of Natural Resources
Department of Social and Health Services
University of Washington, College of Ocean and Fisheries Sciences

Local

Municipality of Metropolitan Seattle (Metro)

PRIVATE FIRMS

Battelle Northwest Laboratories
Cooper Consultants, Inc.
Evans Hamilton, Inc.
EVS Consultants
Northern Technical Services, Inc.
Parametrix, Inc.
URS Engineers

RESEARCH VESSELS

Clifford A. Barnes (UW)
DNR III (WDNR)
Kittiwake (Private)
Liberty (Metro)
McArthur (NOAA)
Harold K. Streeter (NOAA)

TABLE D-2. CHARACTERISTICS OF POSITIONING METHODS USED FOR SAMPLING IN PUGET SOUND[a]

Method	Areas	User Reported Accuracy Ranges (±m)[b] Absolute	Repeatable	Restrictions	Use Among Local Groups
Sextant Angle Resection	Open Areas	100 - 200	<100	Visibility, landmarks	Low
	Nearshore	10 - 20	Similar	<2 - 3 km from shore	Low
Variable Range Radar (VRR)	All	70 - 120	20 - 50	Increases with distance, featureless topography	Medium
Loran-C	Limited	40 - 100	10 - 30	Only in Loran-C mapped areas	Medium
VRR Visual Fixes, Loran-C	Open Areas	30 - 100	20	Repeatability drops in areas with no Loran reception	High
	Nearshore	10 - 40	Similar	<3-4 km from shore, Loran not as critical to repeatability	Medium
Microwave Systems	All	2.5 - 10	1 - 3	Transponder locations	High

[a] As identified through user interviews.

[b] Reported accuracy ranges are consistent with manufacturer claims with the exception that sextant accuracies only achieve highest accuracies near-shore.

Therefore, sextants are used mainly during region characterization studies when location is not critical. When strict absolute accuracy is needed, microwave systems are frequently used. In areas that have been mapped for Loran-C range lines, absolute accuracies as good as or better than VRR can be achieved with Loran-C. As more stations are occupied in these mapped areas, comparisons of Loran-C signals with other fixes help define areas of signal warpage from land or building interactions, increasing absolute accuracy. These systems and their methods are discussed in detail in Appendix C.

CANDIDATE SYSTEM OVERVIEW

Methods other than those commonly used in Puget Sound for station positioning are available. Characteristics, major advantages, and major disadvantages of each method listed below are summarized in this section. Detailed descriptions are presented in Appendix C.

- Multiple horizontal angles

  Theodolite
  Sextant angle resection

- Multiple electronic ranges

  Variable range radar
  Distance-measuring instruments
  Microwave systems
  Loran-C
  Satellite ranging

- Range and angle

  Theodolite and EDMI
  Total station
  Range-azimuth positioning systems.

Multiple Horizontal Angles

Theodolites have the necessary angular acuracies at the anticipated maximum ranges. They are commonly used as surveying instruments and cost $2,000 (30-sec accuarcy) to $4,000 (10-sec accuracy). At least two theodolites, two operators, a siting target on the vessel, and a three-way communications link to coordinate fixes are required (see Appendix C, Figure C-1). Theodolites can be used only during daylight hours of good visibility.

Sextant angle resection techniques offer adequate angular accuracy (± 10 sec) and sextants costs $1,000-$5,000. A three-arm protractor is required for plotting positions. Two oprators should take simultaneous fixes on moving vessels. Because the operators are on board, a separate

communication link is not necessary and they can also serve as crew. However, the method requires highly visible shore targets and is therefore useful only during daylight hours of good visibility. In addition, it is difficult for even an experienced operator to shoot an accurate fix from a moving platform in adverse weather.

Multiple Electronic Ranges

Positioning with multiple electronic VRR ranges will be less accurate over anticipated distances than electronic positioning methods. Equipment costs range from $4,000 to $10,000. Weather and visibility rarely limit use and extra personnel are not needed to help navigate.

Positioning with Electronic Distance-Measuring Instruments (EDMI) offers adequate accuracy but marginal range beyond 3 km (1.9 mi) without multiple prisms. EDMI systems cost $8,000-$15,000 apiece for long-range units and approach $40,000 for systems with prisms. EDMIs require two staffed stations, a three-way communications link to coordinate fixes, and multiple prism assemblies.

Several microwave navigation systems with sufficient accuracy and adequate range are available for $40,000-$90,000. These systems comprise two shore stations and an on-board transmitter. With an additional shore station, the hyperbolic mode can provide multiple user capability. Limitations include geometry of shore stations; vessel position in the coverage area (i.e., crossing angle limitations); and possible interferences from line-of-site obstructions, sea-surface reflective nulls, and land-sea boundaries.

Positioning from Loran-C ranges offers acceptable repeatable accuracy for relocating at station locations for most sampling objectives. Receivers cost $1,000-$4,000 and do not require additional personnel. Limitations include interference in some areas of Puget Sound and the need to initially locate stations with another method.

Transit satellite-based methods currently do not offer sufficient accuracy except with multiple passes, which are impracticable when a sampling station is only briefly occupied. In the future, required accuracies will be achievable using Navstar GPS satellite-based techniques ($10,000-$40,000 for first units; $1,000 for subsequent production models). Independent geosynchronous satellite networks, such as GEOSTAR, may become available at a system interrogator cost of $450 plus a monthly fee. This method is in the early planning stages and recently received FCC approval. Satellite methods do not require additional personnel.

Range and Angle

A theodolite and EDMI could be paired with a communications link for approximately $10,000-$12,000. Total stations developed for this purpose range in cost from $9,000 for a manual station to $15,000-$25,000 for a

fully automated station. Optical and infrared range limitations exist, and the optical components can be operated only during daylight hours of good visibility. The range-azimuth positioning methods examined (see Appendix C) provide sufficient positional accuracy with a single station, and cost between $65,000 for a manual tracking and $70,000-$100,000 for fully automated tracking.

SCREENING CRITERIA

Candidate systems were evaluated for accuracy, flexibility (i.e., range of conditions under which the system can operate, including use for other purposes), portability, reliability, servicing requirements, availability, cost, and convenience. Results of the evaluation are presented in Table D-3. Methods are presented across the top of this table in order of increasing range capability. Methods that could be eliminated from further consideration for some sampling objectives are marked by an asterisk. Limitations that could preclude further consideration included inability to operate at night or in poor visibility (e.g., optical methods) or availability of comparable methods at lower cost with fewer logistical problems (e.g., medium-range systems). The investigator has to determine the project-specific considerations that could be restrictive enough to preclude use of some positioning methods and to determine if the accuracies required for the study can be achieved. For purposes of this evaluation, medium-range sytems were dropped from consideration and optical methods were considered only for nearshore sampling. Remaining positioning methods were reevaluated for range capability, accuracy, availability, capital and operating costs, and merits of use. This information is summarized below.

Range Considerations

Most of the systems noted for restrictive limitations have inadequate range capabilities for visibility and/or distance reasons. At sampling sites farther from land in Puget Sound, VRR will have to be used at a larger scale and a lower accuracy. Sextants and (to a lesser extent) systems that use theodolites may not permit adequate resolution of targets. Lower resolution results in reduced accuracy. The rest of the remaining systems listed in Table D-3 have adequate ranges to cover most areas of Puget Sound (Figure D-1).

Accuracy

Various methods can meet repeatable accuracies of ±20 m (±66 ft). VRR can consistently position within ±20 m (±66 ft) in areas close to shore with supplement from visual fixes and farther from land in areas with good Loran-C reception. Loran-C alone can reposition within ±20 m (±66 ft) in the Elliott Bay area that has recently been mapped with a Loran signal grid. Also, under good conditions, sextants can easily reposition within this distance. Near shore, sextants may approach the accuracy of microwave systems under perfect conditions. Microwave systems, range-azimuth systems,

TABLE D-3. EVALUATION OF NAVIGATION METHODS FOR STATION POSITIONING

	Sextant Angle Resection	Theodolite	Variable Range Radar (VRR)	VRR, Visuals, Loran-C	Total Stations	Microwave Systems	Range-Azimuth Systems	Medium-Range Systems	Loran-C	GEOSTAR	GPS
Accuracy											
Absolute	M-L	H-M	L	M-L	H-M	H	H-M	H-M	M	H	H-M
Repeatable	H-M	H	M	H-M	H	H	H	M	M	H	H-M
Flexibility	M-L*	L*	H	H	M-L*	H-M	M-L*	M-L	H-L	H	H
Portability	H	M	L	H-L	M	M	M	L	H	H-M	H-L
Reliability	H	H-M	H-M	H-M	H-M	H-M	H-M	H-M	H	?	H
Servicing											
Calibration	L	L	H	H-M	M-L	L	L	H-M	H-M	H	H
Maintenance	H	M	H	H	M	H	M	H-M	H	H	H
Availability											
Equipment	H	H	H	H	M	H	M-L	L*	H	?	M
Service	M-L	M	H	H	M-L	M	M-L	M-L	H-M	?	M
Rental	H	H	L	H-L	M-L	H	L	L	H	?	?
Cost	H	H	H-M	H-M	M-L	L	L*	L*	H	H-L	M-L
Convenience	L	L	H	H	L	L	L	L*	H	H	H-L

H = High ranking (adequate, above average, inexpensive, infrequent).

M = Medium ranking (marginal, average, intermediate).

L = Low ranking (not adequate, below average, expensive, frequent).

* = Significant enough limitation to preclude use as a positioning method under some conditions.

? = Not enough information available to evaluate.

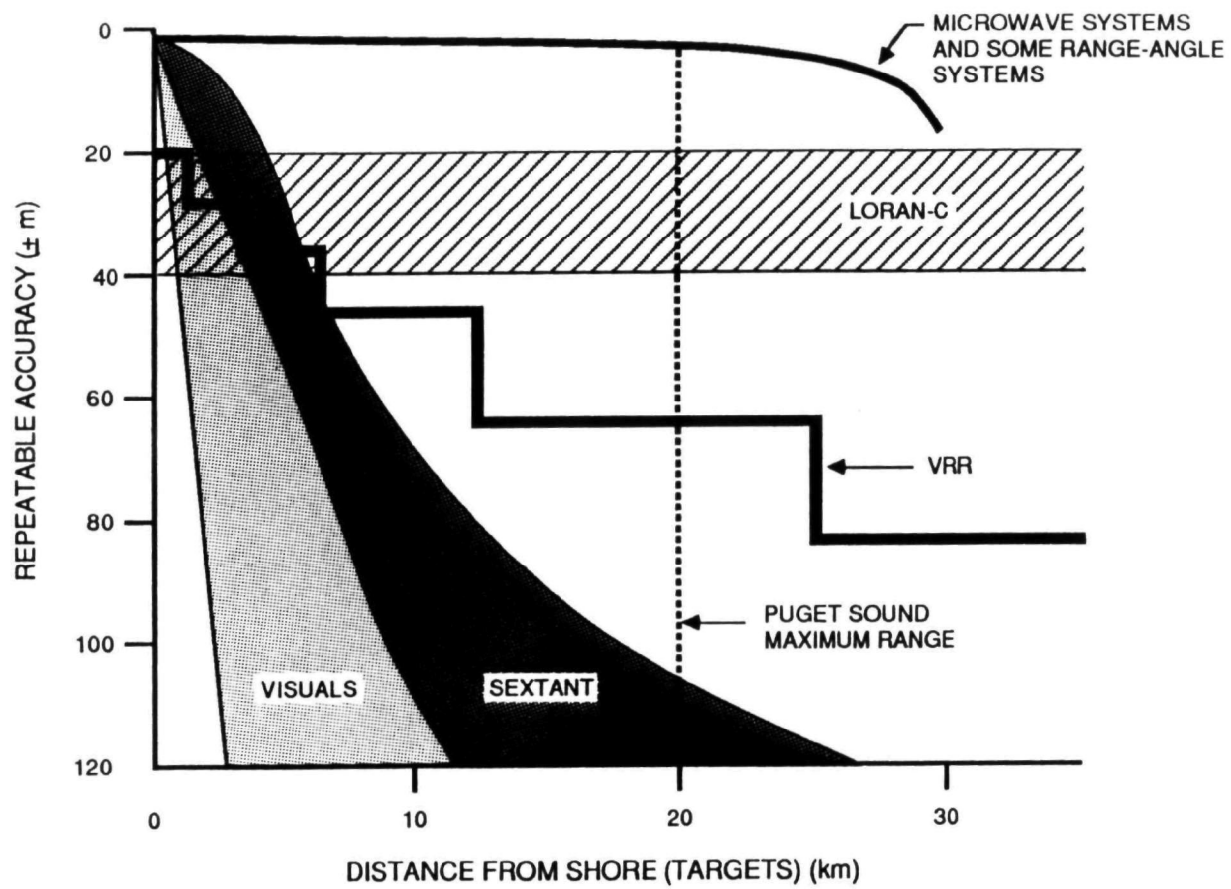

Figure D-1. Approximate accuracy versus distance of some positioning methods.

total stations, and theodolites can reposition a vessel within ±2 m (±6.6 ft). The accuracies of the latter three methods will deteriorate with the visibility. The GPS satellite method accuracies, which vary with satellite code access, are between those of microwave systems and the repeatable accuracy of Loran-C and VRR. GEOSTAR will be almost as accurate as the microwave systems.

The only systems that are capable of obtaining absolute accuracies greater than ±20 m (±66 ft) in most locations are the electronic ranging systems (microwave-based or satellite). Near shore, optical methods and VRR can usually reach these absolute accuracies but are dependent on the available targets and calm, clear conditions.

Availability

The satellite systems will not be usable for an undetermined period of time. GPS does not yet have enough satellites in orbit to give consistent fixes without long time delays. GEOSTAR will not be operational for a few years. The other candidate systems are available.

Capital and Operating Costs

GPS capability is expected to cost from $10,000 to $40,000. Cost of later models is expected to drop to $1,000. Proposed GEOSTAR interrogators are expected to cost $450 plus a monthly use fee. VRR models range from $4,000 to $10,000. Loran-C units cost $1,000-$4,000. Little or no operating cost is associated with these methods and the vessel captain can operate them. Microwave systems typically cost $40,000-$95,000, not including high operating expenses. Range-azimuth systems range from $64,500 to $100,000 and offer greater flexibility for other work and reduced logistical problems. Remaining systems (total stations for $9,000 manual, $15,000-$25,000 automatic; EDMI and theodolites for $10,000-$12,000; and pairs of sextants or theodolites for $4,000-$8,000) are all significantly lower in initial cost. However, these are typically much more labor-intensive and logistically complicated, and can have high operating costs.

Short-term rental or long-term leasing of equipment should be considered as an alternative to purchase of a positioning system. A short-term rental limits cost to only the actual surveying periods and avoids maintenance responsibilities. These arrangements are also attractive during periods of rapid navigation system development (e.g., satellite systems) if such developments indicate that adequate systems will be available in the near future at lower capital costs.

The monthly rental cost of equipment is often set at 10 percent of the suggested retail cost. However, short-term rates for many items are categorized by the number of rental days (e.g., 1-10, 11-90 and 91 plus). Rent/purchase options are available with a set amount of the rental fee (e.g., 50-80 percent) applied to the purchase price when the option is

exercised. Typical rental costs (subject to change) for items of interest are shown in Table D-4. Evaluation of the cost-effectiveness of applicable systems should include rent/lease options as well as procurement. If the user has little experience in a particular system, it may also be appropriate to include the cost of one or more equipment technicians or operators (up to $300 per day plus expenses) to provide reliable navigation data for each survey.

Merits of Use

When repeatable positioning is the primary concern, both Loran-C and VRR methods have adequate accuracies for most sampling objectives. A combination of VRR, visual fixes, Loran-C, and recording station depth will improve repeatable accuracy. Both Loran-C and VRR are routinely carried on research vessels, limiting logistical requirements. The combined method also offers redundancy that minimizes the effect on positioning capability if one of the systems malfunctions.

Sextant fixes are affected by distance, visibility, and rough water. The variability in positioning accuracy across the range of conditions that can be expected make this method questionable for consistent operations across all stations. In good conditions, sextant fixes can improve accuracies over those obtainable for VRR or Loran-C. However, if the investigator is trying to locate specific stations, targets and angles have to be predetermined. Sextants are also valuable as a backup positioning method when the primary system malfunctions.

The shore-based optical methods are not as affected by rough water. Logistical requirements include location of the shore stations and one or two shore station operators. Range-azimuth and total stations offer the advantages of a single shore station and a built-in communications link, which will limit logistical requirements. Two stations have to be staffed and a three-way communications link has to be provided for theodolite positioning. These systems can achieve accuracies of approximately ±2 m (±6.6 ft), but accuracies can deteriorate with visibility and distance to shore for the optical components of these systems.

Microwave systems offer high accuracies [±2 m (±6.6 ft)], but at a high cost. Microwave systems are not limited by visibility restrictions or ranges in Puget Sound. The additional logistical requirements of microwave positioning add complexity to the study design and implementation. When available (approximately 1988), consistent satellite fixes are expected to offer the increased accuracies of microwave sytems with the simplicity of Loran-C.

The radius of the probable sampling area could be reduced by ≤50 m (165 ft) by using a ±2-m absolute accuracy system rather than a ±20-m absolute accuracy system. In deeper waters (e.g., increased sampling area but more homogeneous benthos), the added accuracy would only be advantageous if

TABLE D-4. TYPICAL EQUIPMENT RENTAL COSTS (1985 DOLLARS)

Category		Rental Period 1-10 Days	11-90 Days	91+ Days	Monthly
Theodolites	1"	25-35	18-21	12-15	
	6-10"	22-25	13-15	9-12	
	20"	15-18	10-13	7-10	
EDMIs	1.0-2.0 km	25-30	15-19	10-13	
	2.0-6.0 km	25-65	18-39	15-20	
	>6.0	40-50	25-30	16-22	
Total Stations	Automatic	75-110	45-70	30-60	
	Manual	40-50	25-45	16-20	
Hydrographic Systems					
Trisponder 202		175			2,750
Cubic Auto Tape (3 range)		200	105	90	
Motorola Mini Ranger II (including 2 transponders)		200	105	90	3,750
(additional transponders)		36	18	12	

station grid size or absolute positions were critical. In shallower, near shore waters, the differences in achievable accuracy decrease as resolution of positioning targets improves. However, spatial heterogeneity also increases. The investigator will have to determine if the increase in probable sampling area [now less than 30 m (98 ft) in radius] that results from using less accurate systems will compromise quality of the collected data. If stations were initially located using a highly accurate positioning method (e.g., microwave system) and position coordinates were simultaneously defined in coordinates of a lower accuracy method (e.g., Loran-C, VRR, visual fixes), the station could be reoccupied using the less accurate method, without significant loss in absolute accuracy.

SEDIMENT CONVENTIONALS

FINAL REPORT
TC-3991-04

Puget Sound Estuary Program

RECOMMENDED PROTOCOLS FOR MEASURING CONVENTIONAL SEDIMENT VARIABLES IN PUGET SOUND

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INTRODUCTION

Recommended methods for measuring the following conventional sediment variables in Puget Sound are presented in this chapter:

- Particle size
- Total solids
- Total volatile solids
- Total organic carbon
- Oil and grease
- Total sulfides
- Total nitrogen
- Biochemical oxygen demand
- Chemical oxygen demand.

Each method is based on the results of a workshop and written reviews by representatives from most organizations that fund or conduct environmental research in Puget Sound (Table 1). The purpose of developing these recommended protocols is to encourage all Puget Sound investigators conducting monitoring programs, baseline surveys, and intensive investigations to use standardized methods whenever possible. If this goal is achieved, most data collected in Puget Sound should be directly comparable and thereby capable of being integrated into a sound-wide database. Such a database is necessary for developing and maintaining a comprehensive water quality management program for Puget Sound.

Each recommended protocol describes the use and limitations of the respective variable; the field collection and processing methods; and the laboratory analytical, QA/QC, and data reporting procedures. Each recommended analytical procedure was modified from Plumb (1981). The general collection and holding recommendations for each variable are presented in Table 2.

Although the following protocols are recommended for most studies conducted in Puget Sound, departures from these methods may be necessary to meet the special requirements of individual projects. If such departures are made, however, the funding agency or investigator should be aware that the resulting data may not be comparable with most other data of that kind. In some instances, data collected using different methods may be compared if the methods are intercalibrated adequately.

TABLE 1. CONTRIBUTORS TO THE SEDIMENT CONVENTIONAL PROTOCOLS

Name	Organization
John Armstrong[a]	U.S. EPA
Bob Barrick[a]	Tetra Tech, Inc.
Scott Becker[a,b]	Tetra Tech, Inc.
Gordon Bilyard[a]	Tetra Tech, Inc.
Chuck Boatman[a]	URS Engineers
Eric Crecelius[a]	Battelle Northwest
Joe Cummins	U.S. EPA
Bob Dexter[a]	EVS Consultants
George Ditsworth	U.S. EPA
John Downing[a]	Nortec
Bruce Duncan	U.S. EPA
Mark Fugiel[a]	Am Test, Inc.
Arnold Gahler	U.S. EPA
Carolyn Gangmark	U.S. EPA
Roy Jones	U.S. EPA
Dave Kendall	U.S. COE
Shawn Moore[a]	Am Test, Inc.
Gary Mauseth[a]	Nortec
Mike Nelson[a]	Laucks Testing Labs
Ahmad Nevissi[a]	Univ. of Washington
Wally Triol[a]	Parametrix, Inc.
Frank Urabeck	U.S. COE
Steve Vincent[a]	Weyerhauser Company
Fred Weinmann[a]	U.S. COE
Julia Wilcox[a]	Tetra Tech, Inc.
Carolyn Wilson	U.S. EPA
Jack Word[a]	Evans-Hamilton, Inc.

[a] Attended the workshop held on June 28, 1985.

[b] Workshop moderator.

TABLE 2. RECOMMENDED SAMPLE SIZES, CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES FOR SEDIMENT CONVENTIONAL VARIABLES

Variable	Minimum Sample Size (g)[a]	Container[b]	Preservation	Maximum Holding Time
Particle size	100-150[c]	P,G	Cool, 4° C	6 mo[d]
Total solids	50	P,G	Freeze	6 mo[d]
Total volatile solids	50	P,G	Freeze	6 mo[d]
Total organic carbon	25	P,G	Freeze	6 mo[d]
Oil and grease	100	G only	Cool, 4° C, HCl; Freeze	28 days[d] 6 mo[d]
Total sulfides	50	P,G	Cool, 4° C, 1N zinc acetate	7 days[d]
Total nitrogen	25	P,G	Freeze	6 mo[d]
Biochemical oxygen demand	50	P,G	Cool, 4° C	7 days[e]
Chemical oxgyen demand	50	P,G	Cool, 4° C	7 days[e]

[a] Recommended field sample sizes for one laboratory analysis. If additional laboratory analyses are required (e.g., replicates), the field sample size should be adjusted accordingly.

[b] P = polyethylene, G = glass.

[c] Sandier sediments require larger sample sizes than do muddier sediments.

[d] This is a suggested holding time. No U.S. EPA criteria exist for the preservation of this variable.

[e] This holding time is recommended by Plumb (1981).

COLLECTION OF SURFICIAL SEDIMENTS FOR PHYSICAL AND CHEMICAL VARIABLES

INTRODUCTION

This section describes the protocols required to collect an acceptable subtidal surficial sediment sample for subsequent measurement of physical and chemical variables. This subject has generally been neglected in the past and sampling crews have been given relatively wide latitude in deciding how to collect samples. However, because sample collection procedures influence the results of all subsequent laboratory and data analyses, it is critical that samples be collected using acceptable and standardized techniques.

DESIGN OF SAMPLER

In Puget Sound, the most common sampling device for subtidal surficial sediments is the modified van Veen bottom grab. However, a variety of coring devices is also used. The primary criterion for an adequate sampler is that it consistently collect undisturbed samples to the required depth below the sediment surface without contaminating the samples. An additional criterion is that the sampler can be handled properly on board the survey vessel. An otherwise acceptable sampler may yield inadequate sediment samples if it is too large, heavy, or awkward to be handled properly.

Collection of undisturbed sediment requires that the sampler:

- Create a minimal bow wake 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.

Most modified van Veen grabs have open upper faces that are fitted with rubber flaps. Upon descent, the flaps are forced open to minimize the bow wake, whereas upon ascent, the flaps are forced closed to prevent sample winnowing. Some box corers have solid flaps that are clipped open upon descent and snap shut after the corer is triggered. Although most samplers seal adequately when purchased, the wear and tear of repeated field use eventually reduces this sealing ability. A sampler should therefore be monitored constantly for sample leakage. If unacceptable leakage occurs, the sampler should be repaired or replaced. If a sampler is to be borrowed or leased for a project, its sealing ability should be confirmed prior to sampling. Also, it is prudent to have a backup sampler on board the survey vessel in case the primary sampler begins leaking during a cruise.

The required penetration depth below the sediment surface is a function of the desired sample depth (see Penetration Depth). Generally, it is better to penetrate below the desired sample depth to minimize sample disturbance when the sampling device closes. Penetration depth of most sampling devices varies with sediment character, and generally is greatest in fine sediments and least in coarse sediments. Sampling devices generally rely upon either gravity or a piston mechanism to penetrate the sediment. In both cases, penetration depth can be modified by adding or subtracting weight from the samplers. Thus, it is optimal to use a sampler that has a means of weight adjustment. If a sampler cannot consistently achieve the desired penetration depth, an alternate device should be used.

Once the sampler is secured on board the survey vessel, it is essential that the surface of the sample be made accessible without disturbing the sample. Generally, samplers have hinged flaps on their upper face for this purpose. The opening(s) in the upper face of the sampler should be large enough to allow easy subsampling of the sediment surface. If an opening is too small, the sample may be disturbed as the scientific crew member struggles to take a subsample.

PENETRATION DEPTH

For characterizing surficial sediments in Puget Sound, it is recommended that the upper 2 cm of the sediment column be evaluated. When collecting the upper 2 cm of sediment, it is recommended that a minimum penetration depth of 4-5 cm be achieved for each acceptable sample.

Although the 2-cm specification is arbitrary, it will ensure that:

- Relatively recent sediments are sampled
- Adequate volumes of sediments can be obtained readily for laboratory analyses
- Data from different studies can be compared validly.

Sampling depths other than 2 cm may be appropriate for specific purposes. For example, the upper 1 cm of sediment may be required to determine the age of the most recently deposited sediments. By contrast, a sample depth much greater than 2 cm may be required to evaluate the vertical profile of sediment characteristics or to determine depth-averaged characteristics prior to dredging. If a sampling depth other than 2 cm is used, comparisons with data from 2-cm deep samples may be questionable.

OPERATION OF SAMPLER

The sampling device should be attached to the hydrowire using a ball-bearing swivel. The swivel will minimize the twisting forces on the sampler during deployment and ensure that proper contact is made with the bottom.

For safety, the hydrowire, swivel, and all shackles should have a load capacity at least three times greater than the weight of a full sampler.

The sampler should be lowered through the water column at a controlled speed of approximately 1 ft/sec. Under no circumstances should the sampler be allowed to "free fall" to the bottom, as this may result in premature triggering, an excessive bow wake, or improper orientation upon contact with the bottom. The sampler should contact the bottom gently and only its weight or piston mechanism should be used to force it into the sediment.

After the sediment sample is taken, the sampler should be raised slowly off the bottom and then retrieved at a controlled speed of approximately 1 ft/sec. Before the sampler breaks the water surface, the survey vessel should head into the waves (if present) to minimize vessel rolling. This maneuver will minimize swinging of the sampler after it breaks the water surface. If excessive swinging occurs or if the sampler strikes the vessel during retrieval, extra attention should be paid to evaluating sample disturbance when judging sample acceptability.

The sampler should be secured immediately after it is brought on board the survey vessel. If the sampler tips or slides around before being secured, extra attention should be paid to evaluating sample disturbance.

SAMPLE ACCEPTABILITY CRITERIA

After the sampler is secured on deck, the sediment sample should be inspected carefully before being accepted. The following acceptability criteria should be satisfied:

- The sampler is not over-filled with sample so that the sediment surface is pressed against the top of the sampler
- Overlying water is present (indicates minimal leakage)
- The overlying water is not excessively turbid (indicates minimal sample disturbance)
- The sediment surface is relatively flat (indicates minimal disturbance or winnowing)
- The desired penetration depth is achieved (i.e., 4-5 cm for a 2-cm deep surficial sample).

If a sample does not meet all criteria, it should be rejected.

SAMPLE COLLECTION

After a sample is judged acceptable, the following observations should be noted on the field log sheet:

- Station location
- Depth

- Gross characteristics of the surficial sediment
  - Texture
  - Color
  - Biological structures (e.g., shells, tubes, macrophytes)
  - Presence of debris (e.g., wood chips, wood fibers, human artifacts)
  - Presence of oily sheen
  - Odor (e.g., hydrogen sulfide, oil, creosote)
- Gross characteristics of the vertical profile
  - Changes in sediment characteristics
  - Presence and depth of redox potential discontinuity (rpd) layer
- Penetration depth
- Comments related to sample quality
  - Leakage
  - Winnowing
  - Disturbance.

Before subsamples of the surficial sediments are taken, the overlying water must be removed. The preferred method of removing this water is by slowly siphoning it off near one side of the sampler. Methods such as decanting the water or slightly cracking the grab to let the water run out are not recommended, as they may result in unacceptable disturbance or loss of fine-grained surficial sediment and organic matter.

Once the overlying water has been removed, the surficial sediment can be subsampled. It is recommended that subsamples be taken using a flat scoop shaped like a coal shovel. The shoulders of the scoop should be 2 cm high. This device will allow a relatively large subsample to be taken accurately to a depth of 2 cm. Coring devices are not recommended because generally they collect small amounts of surficial sediment and therefore require repeated extractions to obtain a sufficient volume of material for analysis of conventional sediment variables. A curved scoop is not recommended because it does not sample a uniform depth. Because accurate and consistent subsampling requires practice, it is advisable that an experienced person perform this task.

When subsampling surficial sediments, unrepresentative material should be removed in the field under the supervision of the chief scientist and noted on the field log sheet. The criteria used to determine representativeness should be determined prior to sampling.

Finally, if samples are to be analyzed for trace metals or priority pollutant organic compounds, sample contamination during collection must be avoided. All sampling equipment (i.e., siphon hoses, scoops, containers) should be made of noncontaminating material and should be cleaned appropriately before use. Samples should not be touched with ungloved fingers. In addition, potential airborne contamination (e.g., stack gases, cigarette smoke) should be avoided. Detailed guidance for preventing sample

contamination is given in the protocols for metals and organic compounds in other chapters of this notebook.

PARTICLE SIZE

USE AND LIMITATIONS

Particle size is used to characterize the physical characteristics of sediments. Because particle size influences both chemical and biological variables, it can be used to normalize chemical concentrations according to sediment characteristics and to account for some of the variability found in biological assemblages. Particle size is also an important variable for marine engineering purposes. In addition to Plumb (1981), a variety of other references discuss the uses and measurement of particle size (e.g., Krumbein and Pettijohn 1938; Folk 1968; Buchanan 1984).

Particle size can be characterized in a wide range of detail. The grossest divisions that generally are considered useful for characterizing particle size distributions are percentages of gravel, sand, silt, and clay. However, each of these size fractions can be subdivided further so that additional characteristics of the size distribution (e.g., mean diameter, skewness, kurtosis) can be determined.

Particle size determinations can either include or exclude organic material. If organic material is removed prior to analysis, the "true" (i.e., primarily inorganic) particle size distribution is determined. If organic material is included in the analysis, the "apparent" (i.e., organic plus inorganic) particle size distribution is determined. Because true and apparent distributions may differ, detailed comparisons between samples analyzed by these different methods are questionable. It is therefore desirable that all samples within each study (at a minimum) and among different studies (if possible) be analyzed using only one of these two methods.

FIELD PROCEDURES

Collection

Samples can be collected in glass or plastic containers. A minimum sample size of 100-150 g is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

Processing

Samples should be stored at 4° C, and can be held for up to 6 mo before analysis. Samples must not be frozen or dried prior to analysis, as either process may change the particle size distribution.

LABORATORY PROCEDURES

Analytical Procedures

- Equipment
  - Sieve shaker
    Ro-Tap or equivalent
  - Drying oven
  - Constant temperature bath
  - Analytical balance
    0.1 mg accuracy
  - Desiccator
  - Clock
    With second hand
  - Standard sieves
    Appropriate mesh sizes
  - Sieve pan and top
  - Sieve brush
  - Funnel
  - 1-L graduated cylinders
  - 50-mL beakers
  - 20-mL pipets
  - Water pique or squirt bottle
  - Glossy paper
  - Dispersant
    1 percent sodium hexametaphosphate = 1 percent commercially available Calgon
  - Distilled water.

- Sample preparation
  - Allow samples to warm to room temperature.
  - Homogenize each sample mechanically, incorporating any overlying water.
  - Remove a representative aliquot (approximately 25 g) and analyze for total solids content. This information can be used to estimate the dry weight of the aliquot used for particle size analysis. The efficiency of the entire analysis can then be evaluated by adding the dry weights of all sample fractions and comparing this sum with the estimated dry weight of the original aliquot.
  - Remove a second representative aliquot for wet sieving. The aliquot can range from 20 g for muddy sediments to 100 g for sandy sediments. The critical factor for sample size determination is

the weight of fine-grained material that will be used for the pipet analysis. Ideally the total dry weight of fine-grained material in the 1-L graduated cylinder should equal approximately 15 g. However, total weights between 5 and 25 g are considered acceptable. Total weights outside this range are not considered acceptable and it is recommended that aliquot size be modified to bring the amount of fine-grained material into the acceptable range.

- Weigh the wet sample to the nearest 0.01 g.

- Organics oxidation - this step removes organic material from the sample. It is optional and depends upon the objectives of each study.
  - Place the sediment sample in a large beaker ($\geq$ 2 L).
  - Add 20 mL of 10 percent hydrogen peroxide solution and mix.
  - Let the sample stand until frothing stops.
  - Once frothing stops, add an additional 10 mL of hydrogen peroxide solution.
  - Continue adding 10-mL portions of hydrogen peroxide solution until no frothing occurs on addition.
  - Boil the sample to remove any excess hydrogen peroxide.
  - Be careful that material is not lost from the beaker during frothing and boiling.

- Wet-sieving - this step separates the sample into size fractions greater than 62.5 um (i.e., sand and gravel) and less than 62.5 um (i.e., silt and clay)
  - Place the 62.5-um (4 phi) sieve in a funnel, with a 1-L graduated cylinder underneath. Moisten the sieve using a light spray of distilled water.
  - Place the sample in a beaker, add 20-30 mL of distilled water, and stir to suspend fine-grained material.
  - Pour the sample into the sieve and thoroughly rinse the beaker and stirrer with distilled water.
  - Wash the sediment on the sieve with distilled water using a water pique or squirt bottle having low water pressure. Aggregates can be gently broken using a rubber policeman.
  - Continue wet sieving until only clear water passes through the sieve. Try to ensure that the rinsate does not exceed approximately 950 mL. This can generally be accomplished by sieving a sample quantity that is not too large and by efficient use of the rinse water. Both of these techniques may require experimentation before routine wet sieving is started.

- Gravel-sand fraction - this fraction is subdivided further by mechanically dry sieving it through a graded series of screens.
  - Wash the coarse fraction into a preweighed 50-mL beaker using distilled water. Rinse the sieve thoroughly.

- Dry the coarse fraction to constant weight at 90 ± 2° C. The drying temperature is less than 100° C to prevent boiling and potential loss of sample.
- Cool the sample to room temperature in a desiccator.
- Weigh the cooled sample to the nearest 0.1 mg.
- Set up a nest of sieves that will divide the coarse fraction into the desired number of subfractions. Set up the sieves in a graded series of mesh sizes, with the coarsest mesh on top and the finest mesh on the bottom. The bottom sieve always should have a mesh size of 62.5 um (4 phi). Place a solid pan on the bottom of the stack and a lid on top of the stack. At a minimum, the coarse fraction should be separated into gravel and sand fractions, using a sieve with a mesh size of 2 mm (-1 phi).
- Add the sample to the uppermost sieve. Complete transfer can be ensured by using a sieve brush to remove any material adhering to the beaker. The sieve brush can also be used to gently break up aggregated sediment.
- Shake mechanically for exactly 15 min using the Ro-Tap (or equivalent). A shaker having an automatic timer is preferable.
- After shaking, empty the contents of each sieve onto a glossy piece of paper (e.g., wax paper). To empty a sieve, invert it and tap it on the table several times while ensuring that all edges hit the table at the same time. If the sieve is not tapped evenly, the meshes may be distorted. After tapping the sieve, ensure complete removal of the sample by brushing the back of the screen. After brushing the back of the screen, turn the sieve over and brush out any particles adhering to the sides of the sieve or the inside of the screen.
- Add the fraction that passed through the bottom sieve (e.g., 4 phi) and was retained by the solid pan to the silt-clay fraction of that sample.
- Weigh each remaining size fraction to the nearest 0.1 mg.
- Sum the weights of all size fractions and compare the result with the initial weight of the coarse fraction. Losses and inaccuracies should be less than 5 percent of the initial weight. Losses and inaccuracies tend to increase with increasing number of fractions.
- Large amounts of organically derived fragments (e.g., wood debris, grass, shells) or any unusual material in any size fraction should be noted on the laboratory log sheet.

- Silt-clay fraction - this fraction is subdivided further using a pipet technique that depends upon the differential settling rates of different particles. Because additions to this fraction may be made after mechanical sieving of the gravel-sand fraction (see above), it is recommended that the silt-clay analysis for each sample not be conducted until the gravel-sand analysis has been completed.
  - Add 10 mL of the dispersant to 990 mL of distilled water. Determine the weight of dispersant in a 20-mL aliquot of this

mixture by pipeting a 20-mL aliquot into each of five tared beakers, drying the samples to constant weight at 90 ± 2° C, cooling the samples in a desiccator, weighing the cooled samples, and calculating the mean weight of dispersant in the five samples. This weight multiplied by the number of 10-mL additions of dispersant to each sample will be subtracted from the weight of each sediment fraction at the end of the pipet analysis.

- Add 10 mL of the dispersant to each sample suspension in the 1-L graduated cylinders.
- Mix each suspension by either stoppering and inverting the cylinder or by using the up and down motion of a perforated disc plunger.
- Allow the mixed suspension to stand for 2-3 h and check for signs of flocculation. Flocculation can be recognized by a curdling and rapid settling of lumps of particles or by the presence of a thick soupy layer on the bottom of the cylinder passing abruptly into clear water above.
- If flocculation occurs, add dispersant in 10-mL increments until no noticeable flocculation occurs. Record the volume of dispersant added.
- When ready to conduct the pipet analysis, bring the sample volume to 1 L by adding distilled water, mix the suspension thoroughly, and place the cylinder in a constant-temperature water bath. If the volume is greater or less than 1 L, the factor for converting the weight of the sediment in each 20-mL aliquot to that in the total volume must be modified accordingly.
- After 20 sec, withdraw a 20-mL aliquot from a depth of 20 cm below the surface of the suspension using a pipet. The pipet should be marked for the specified sampling depths and should be inserted vertically into the settling cylinder when the aliquot is taken. A suction bulb may be used on the open end of the pipet to facilitate sampling. It is critical that the suspension be disturbed as little as possible when pipet aliquots are taken.
- Transfer the 20-mL aliquot to a preweighed 50-mL beaker. Rinse the pipet into the beaker using 20 mL of distilled water.
- Withdraw 20-mL aliquots at a depth of 10 cm below the surface of the suspension at the appropriate time(s) listed in Table 3. A formula for calculating withdrawal times is given by Folk (1968) and Buchanan (1984). If a withdrawal is missed, the suspension can be stirred again and the missed withdrawal can be taken at the appropriate time after settling begins. It is not necessary to withdraw the initial 20-mL aliquot when this corrective action is conducted.
- Transfer these additional 20-mL aliquots to 50-mL preweighed beakers, each time rinsing the pipet into the respective beaker using 20 mL of distilled water
- Dry all aliquots to constant weight at 90 ± 2° C. A drying temperature less than 100° C is used to prevent boiling and potential loss of sample.

TABLE 3. WITHDRAWAL TIMES FOR PIPET ANALYSIS AS A FUNCTION OF PARTICLE SIZE AND WATER TEMPERATURE[a,b]

Diameter Finer than (phi)	Diameter Finer than (um)	Withdrawal Depth (cm)	Elapsed Time for Withdrawal of Sample in Hours (h), Minutes (m), and Seconds (s)						
			18° C	19° C	20° C	21° C	22° C	23° C	24° C
4.0	62.5	20	20s	20s	20s	20s	20s	20s	20s
5.0	31.2	10	2m0s	1m57s	1m54s	1m51s	1m49s	1m46s	1m44s
6.0	15.6	10	8m0s	7m48s	7m36s	7m25s	7m15s	7m5s	6m55s
7.0	7.8	10	31m59s	31m11s	30m26s	29m41s	28m59s	28m18s	27m39s
8.0[c]	3.9	10	2h8m	2h5m	2h2m	1h59m	1h56m	1h53m	1h51m
9.0	1.95	10	8h32m	8h18m	8h6m	7h56m	7h44m	7h32m	7h22m
10.0	0.98	10	34h6m	33h16m	32h28m	31h40m	30h56m	30h12m	29h30m

[a] Modified from Plumb (1981).

[b] It is critical that temperature be held constant during the pipet analysis.

[c] Breakpoint between silt and clay.

- Cool dried samples to room temperature in a desiccator.
- Weigh cooled samples to the nearest 0.1 mg.

- Calculations
  - The total weight of a phi-size interval in the 1-L graduated cylinder is determined as follows:

$$\text{Phi weight (g dry weight)} = 50[(A-C)-(B-C)]$$

Where:

A = weight (g) of residue in a 20-mL aliquot for a given phi-size boundary
B = weight (g) residue in a 20-mL aliquot for the next larger phi-size boundary
C = mean weight (g) of dispersant in a 20-mL aliquot.

QA/QC Procedures

It is critical that each sample be homogenized thoroughly in the laboratory before a subsample is taken for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

After dry-sieving a sample, all material must be removed from the sieve. This can be accomplished by tapping the rim of the sieve evenly on a hard surface and by brushing the screen.

The total amount of fine-grained material used for pipet analysis should be 5-25 g. If more material is used, particles may interfere with each other during settling and the possibility of flocculation may be enhanced. If less material is used, the experimental error in weighing becomes large relative to the sample size.

Before pipet extractions can be made, the sample must be homogenized thoroughly within the settling cylinder. Once the pipet analysis begins, the settling cylinders must not be disturbed, as this will alter particle settling velocities. Care must be taken to disturb the sample as little as possible when pipet extractions are made.

After a pipet extract has been transferred to a drying beaker, any sample adhering to the inside of the pipet must be removed. This can be accomplished by drawing 20 mL of distilled water into the pipet and adding this rinse water to the drying beaker.

Dried samples should be cooled in a desiccator and held there until they are weighed. If a desiccator is not used, the sediment will accumulate ambient moisture and the sample weight will be overestimated. A color-indicating desiccant is recommended so that spent desiccant can be detected

easily. Also, the seal on the desiccator should be checked periodically, and, if necessary, the ground glass rims should be greased or the "O" rings should be replaced.

It is recommended that triplicate analyses be conducted on one of every 20 samples, or on one sample per batch if less than 20 samples are analyzed. It is also recommended that the analytical balance, drying oven, and temperature bath be inspected daily and calibrated at least once per week.

DATA REPORTING REQUIREMENTS

The weight of each sediment fraction should be reported to the nearest 0.0001 g dry weight. The laboratory should report the results of all samples analyzed (including QA replicates) and should note any problems that may have influenced data quality.

TOTAL SOLIDS

USE AND LIMITATIONS

Total solids are the organic and inorganic materials remaining after a sample has been dried completely. This variable is commonly used to convert sediment concentrations of substances from a wet-weight to a dry-weight basis. It typically is measured in conjunction with other variables.

Total solids values are operationally defined, because results depend on drying temperatures. For example, temperature-dependent weight losses occur from volatilization of organic matter, mechanically occluded water, water of crystallization, and gases from heat-induced chemical decomposition. By contrast, weight gains may result from oxidation processes. To provide data that are comparable among different studies, it is therefore critical that drying temperatures be standardized.

FIELD PROCEDURES

Collection

Samples can be collected in glass or plastic containers. Samples can be collected in the same containers as samples for other variables, if total solids is to be measured in conjunction with those variables. A minimum sample size of 50 g is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

Processing

Samples should be stored frozen and can be held for up to 6 mo under that condition.

LABORATORY PROCEDURES

Analytical Procedures

- Equipment
  - Muffle furnace
    550° C capacity
  - Drying oven
  - Desiccator

- Analytical balance
  0.01 g accuracy
- 100-mL evaporating dishes
  Porcelain, platinum, or Vycor.

- Equipment preparation
  - Ignite clean evaporating dishes at 550 ± 10° C for 1 h in a muffle furnace to remove any remaining organic material.
  - Cool ignited dishes to room temperature in a desiccator.
  - Weigh each cooled dish to the nearest 0.01 g and store in the desiccator.

- Sample preparation
  - Allow frozen sediment samples to warm to room temperature
  - Homogenize each sample mechanically, incorporating any overlying water.
  - Transfer a representative subsample (approximately 25 g) to a preweighed evaporation dish.
  - Weigh the undried sample to the nearest 0.01 g.

- Analytical procedures
  - Dry the sample to constant weight at 103 ± 2° C.
  - Cool the dried sample to room temperature in a desiccator.
  - Weigh the cooled sample to the nearest 0.01 g.

- Calculations
  - Total solids content is determined as follows:

$$\text{Percent solids} = \frac{(A-B)(100)}{C-B}$$

Where:

A = weight (g) of dish and dry sample residue
B = weight (g) of dish
C = weight (g) of dish and wet sample.

QA/QC Procedures

It is critical that each sample be thoroughly homogenized in the laboratory before a subsample is taken for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

Evaporating dishes must be ignited at 550° C before being used for total solids analysis. This step ensures that dishes are free from organic contaminants.

Dried samples should be cooled in a desiccator and held there until they are weighed. If a desiccator is not used, the sediment will accumulate

ambient moisture and the sample weight will be overestimated. A color-indicating desiccant is recommended so that spent desiccant can be detected easily. Also, the seal on the desiccator should be checked periodically and, if necessary, the ground glass rims should be greased or the "O" rings should be replaced.

It is recommended that triplicate analyses be conducted on one of every 20 samples or on one sample per batch if less than 20 samples are analyzed. It is also recommended that the analytical balance and drying oven be inspected daily and calibrated at least once per week.

DATA REPORTING REQUIREMENTS

Total solids should be reported as a percentage of the wet weight of the sample to the nearest 0.1 unit. The laboratory should report the results of all samples analyzed (including QA replicates) and should note any problems that may have influenced sample quality.

TOTAL VOLATILE SOLIDS (TVS)

USE AND LIMITATIONS

Total volatile solids represent the fraction of total solids that are lost on ignition at a higher temperature than that used to determine total solids. Total volatile solids is used as a crude estimate of the amount of organic matter in the total solids.

Total volatile solids is operationally defined by the ignition temperature. Total volatile solids content does not always represent the organic content of a sample because some organic material may be lost at the drying temperature and some inorganic material (e.g., carbonates, chlorides) may be lost at the ignition temperature. Because of the temperature dependence of total volatile solids, valid interstudy comparisons require the use of standardized drying and ignition temperatures.

FIELD PROCEDURES

Collection

Samples can be collected in glass or plastic containers. A minimum sample size of 50 g is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

Processing

Samples should be stored frozen and can be held for up to 6 mo under that condition.

LABORATORY PROCEDURES

Analytical Procedures

- Equipment
  - Muffle furnace
    550° C capacity
  - Drying oven
  - Desiccator
  - Analytical balance
    0.01 g accuracy
  - 100-mL evaporating dishes
    Porcelain, platinum, or Vycor.

- Equipment preparation
  - Ignite clean evaporating dishes at 550 ± 10° C for 1 h in a muffle furnace to remove any remaining organic material.
  - Cool ignited dishes to room temperature in a desiccator.
  - Weigh each cooled dish to the nearest 0.01 g and store in the desiccator.

- Sample preparation
  - Allow frozen sediment samples to warm to room temperature.
  - Homogenize each sample mechanically, incorporating any overlying water.
  - Transfer a representative subsample (approximately 25 g) to a preweighed evaporating dish.

- Analytical procedures
  - Dry the sample to constant weight at 103 ± 2° C.
  - Cool the dried sample to room temperature in a desiccator.
  - Weigh the cooled sample to the nearest 0.01 g.
  - Ignite the sample at 550 ± 10° C to constant weight. Make sure that the samples do not flare up when placed in the oven, as sediment may be lost from the crucibles. If sample flashing is a problem, it is recommended that the muffle furnace be cooler than 550° C when samples are placed inside, and that the temperature gradually be increased to 550° C.
  - Weigh each cooled sample to the nearest 0.01 g.

- Calculations
  - TVS content is determined as follows:

$$\text{Percent TVS} = \frac{(A-C)100}{A-B}$$

Where:

A = weight (g) of dish and dry sample residue
B = weight (g) of evaporation dish
C = weight (g) of dish and ignition residue.

QA/QC Procedures

It is critical that each sample be thoroughly homogenized in the laboratory before a subsample is taken for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

Evaporating dishes (or crucibles) must be ignited at 550° C before being used for total volatile solids analysis. This step ensures that the dishes are free from volatile contaminants.

Dried and combusted samples should be cooled in a desiccator and held there until they are weighed. If a desiccator is not used, the sediment will accumulate ambient moisture and the sample weight will be overestimated. A color-indicating desiccant is recommended so that spent desiccant can be detected easily. Also, the seal on the desiccator should be checked periodically and, if necessary, the ground glass rims should be greased or the "O" rings should be replaced.

It is recommended that triplicate analyses be conducted on one of every 20 samples or on one sample per batch if less than 20 samples are analyzed. It is also recommended that the analytical balance, drying oven, and muffle furnace be inspected daily and calibrated at least once per week.

DATA REPORTING REQUIREMENTS

Total volatile solids should be reported as a percentage of the dry weight of the uncombusted sample to the nearest 0.1 unit. The laboratory should report the results of all samples analyzed (including QA replicates) and should note any problems that may have influenced data quality.

TOTAL ORGANIC CARBON (TOC)

USE AND LIMITATIONS

Total organic carbon is a measure of the total amount of nonvolatile, volatile, partially volatile, and particulate organic compounds in a sample. Total organic carbon is independent of the oxidation state of the organic compounds and is not a measure of the organically bound and inorganic elements that can contribute to the biochemical and chemical oxygen demand tests.

Because inorganic carbon (e.g., carbonates, bicarbonates, free CO_2) will interfere with total organic carbon determinations, samples should be treated to remove inorganic carbon before being analyzed.

FIELD PROCEDURES

Collection

Samples can be collected in glass or plastic containers. A minimum sample size of 25 g is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

Processing

Samples should be stored frozen and can be held for up to 6 mo under that condition. Excessive temperatures should not be used to thaw samples.

LABORATORY PROCEDURES

Analytical Procedures

- Equipment
  - Induction furnace
    e.g., Leco WR-12, Dohrmann DC-50, Coleman CH analyzer, Perkin Elmer 240 elemental analyzer, Carlo-Erba 1106
  - Analytical balance
    0.1 mg accuracy
  - Desiccator
  - Combustion boats
  - 10 percent hydrochloric acid (HCl)
  - Cupric oxide fines (or equivalent material)
  - Benzoic acid or other carbon source as a standard.

- Equipment preparation
  - Clean combustion boats by placing them in the induction furnace at 950° C. After being cleaned, combustion boats should not be touched with bare hands.
  - Cool boats to room temperature in a desiccator.
  - Weigh each boat to the nearest 0.1 mg.

- Sample preparation
  - Allow frozen samples to warm to room temperature.
  - Homogenize each sample mechanically, incorporating any overlying water.
  - Transfer a representative aliquot (5-10 g) to a clean container.

- Analytical procedures
  - Dry samples to constant weight at 70 ± 2° C. The drying temperature is relatively low to minimize loss of volatile organic compounds.
  - Cool dried samples to room temperature in a desiccator.
  - Grind sample using a mortar and pestle to break up aggregates.
  - Transfer a representative aliquot (0.2-0.5 g) to a clean, preweighed combustion boat.
  - Determine sample weight to the nearest 0.1 mg.
  - Add several drops of HCl to the dried sample to remove carbonates. Wait until the effervescing is completed and add more acid. Continue this process until the incremental addition of acid causes no further effervescence. Do not add too much acid at one time as this may cause loss of sample due to frothing. Exposure of small samples (i.e., 1-10 mg) having less than 50 percent carbonate to an HCl atmosphere for 24-48 h has been shown to be an effective means of removing carbonates (Hedges and Stern 1984). If this method is used for sample sizes greater than 10 mg, its effectiveness should be demonstrated by the user.
  - Dry the HCl-treated sample to constant weight at 70 ± 2° C.
  - Cool to room temperature in a desiccator.
  - Add previously ashed cupric oxide fines or equivalent material (e.g., alumina oxide) to the sample in the combustion boat.
  - Combust the sample in an induction furnace at a minimum temperature of 950 ± 10° C.

- Calculations
  - If an ascarite-filled tube is used to capture CO_2, the carbon content of the sample can be calculated as follows:

$$\text{Percent carbon} = \frac{A(0.2729)(100)}{B}$$

Where:

A = the weight (g) of CO_2 determined by weighing the ascarite tube before and after combustion
B = dry weight (g) of the unacidified sample in the combustion boat
0.2729 = the ratio of the molecular weight of carbon to the molecular weight of carbon dioxide

A silica gel trap should be placed before the ascarite tube to catch any moisture driven off during sample combustion. Additional silica gel should be placed at the exit end of the ascarite tube to trap any water that might be formed by reaction of the trapped CO_2 with the NaOH in the ascarite.

- If an elemental analyzer is used, the amount of CO_2 will be measured by a thermal conductivity detector. The instrument should be calibrated daily using an empty boat blank as the zero point and at least two standards. Standards should bracket the expected range of carbon concentrations in the samples.

QA/QC Procedures

It is critical that each sample be thoroughly homogenized in the laboratory before a subsample is taken for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

Dried samples should be cooled in a desiccator and held there until they are weighed. If a desiccator is not used, the sediment will accumulate ambient moisture and the sample weight will be overestimated. A color-indicating desiccant is recommended so that spent desiccant can be detected easily. Also, the seal on the desiccator should be checked periodically and, if necessary, the ground glass rims should be greased or the "O" rings should be replaced.

It is recommended that triplicate analyses be conducted on one of every 20 samples, or on one sample per batch if less than 20 samples are analyzed. A method blank should be analyzed at the same frequency as the triplicate analyses. The analytical balance should be inspected daily and calibrated at least once per week. The carbon analyzer should be calibrated daily with freshly prepared standards. A standard reference material should be analyzed at least once for each major survey.

DATA REPORTING REQUIREMENTS

Total organic carbon should be reported as a percentage of the dry weight of the unacidified sample to the nearest 0.1 unit. The laboratory should report the results of all samples (including QA replicates, method

blanks, and standard reference measurements) and should note any problems that may have influenced sample quality. The laboratory should also provide a summary of the calibration procedure and results (e.g., range covered, regression equation, coefficient of determination).

OIL AND GREASE (FREON-EXTRACTABLE)

USE AND LIMITATIONS

Oil and grease tests measure all material recovered as a substance soluble in a nonpolar solvent (e.g., Freon) under acidic conditions. Oil and grease includes such compounds as hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases, and related industrial compounds.

In addition to oil and grease, the solvent may dissolve other kinds of substances, such as sulfur compounds, organic dyes, and chlorophyll. Oil and grease is therefore operationally defined by the kind of solvent and analytical methods used. Standardized procedures are essential for valid interstudy comparisons. Because oil and grease values obtained using the gravimetric technique sometimes do not correlate with values obtained using the IR technique, it is recommended that a single technique be used within each study (at a minimum) and among different studies (if possible).

FIELD PROCEDURES

Collection

Samples should be collected only in glass containers having TFE-lined lids. Although aluminum-lined lids can be used, seawater eventually will corrode the aluminum. Before being used, containers and lids should first be washed with a warm aqueous detergent mixture and then, in sequence, thoroughly rinsed with hot tap water, rinsed at least twice with distilled water, rinsed once with Freon (i.e., 1,1,2-trichloro-1,2,2-trifluoroethane, and dried at 105 $\pm$ 2° C for 30 min. A minimum sample size of 100 g is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

Processing

If samples cannot be analyzed within 24 h, they can be preserved with approximately 1 mL of concentrated hydrochloric acid (HCl) per 80 g of sample. Acid-preserved samples should be stored at 4° C, and can be held for up to 28 days in that condition. Although U.S. EPA has not established a recommeded maximum holding time for oil and grease in sediments, 28 days is consistent with the recommended holding time for acid-preserved water samples. Samples can also be preserved by freezing at -20° C, and can be held under that condition for up to 6 mo. Samples must be kept field-moist during storage because they may lose apparent oil and grease as a result of drying.

LABORATORY PROCEDURES

Analytical Procedures

- Equipment
  - Infrared spectrophotometer
    IR technique only
  - Analytical balance
    Gravimetric technique only, 0.1 mg accuracy
  - Extraction apparatus, Soxhlet
  - Vacuum pump or other source of vacuum
  - Extraction thimble, paper
  - Concentrated hydrochloric acid (HCl) or concentrated sulfuric acid (H_2SO_4)
  - Magnesium sulfate monohydrate
    Prepare $MgSO_4 \cdot H_2O$ by drying a thin layer of $MgSO_4 \cdot 7H_2O$ overnight in an oven at 103° C
  - Freon (1,1,2-trichloro-1,2,2,-trifluoroethane), boiling point 47° C
    The solvent should leave no measurable residue on evaporation; redistill if necessary
  - Grease-free cotton
    Extract nonabsorbent cotton using Freon
  - Oil reference standard
    If the identity of oil and grease in a sample is unknown, a mixture of 15.0 mL n-hexadecane, 15.0 mL isooctane, and 10.0 mL chlorobenzene should be used as the standard. This is the same reference oil used for water samples in U.S. EPA Method 413.2 (U.S. EPA 1983). If the identity of oil and grease is known, the standard can be comprised of the same substance as that in the sample.

- Sample preparation
  - Allow samples to warm to room temperature.
  - Homogenize each sample mechanically, incorporating any overlying water.
  - Remove a representative aliquot (approximately 25 g) and analyze it for total solids content.
  - Remove a representative aliquot (approximately 20 g) and weigh it to the nearest 0.1 mg.
  - Transfer the weighed aliquot to a 150-mL beaker for oil and grease analysis.

- Oil and grease extraction
  - Acidify the sample to pH = 2 using concentrated HCl or concentrated H_2SO_4.
  - Add 25 g $MgSO_4 \cdot H_2O$ to the acidified sediment sample. Stir to make a uniformly smooth paste that is spread on the beaker wall. Allow to stand 15-30 min until solidified.

- Following solidification, remove the solids and grind in a porcelain mortar. The use of a desiccated, uniformly ground sample improves the efficiency of the extraction process.
- Add the ground sample to a paper extraction thimble. The beaker and mortar should be wiped with a small piece of filter paper that has been soaked in Freon. Add the filter paper to the paper thimble.
- Fill the thimble with glass wool or small glass beads. Extract the prepared sample using Freon in a Soxhlet apparatus at a rate of 20 cycles/h for 4 h. Stir at least twice during extraction to prevent channeling. If the final extract is turbid, filter the sample through grease-free cotton into a clean flask. Rinse the initial sample container and the cotton with Freon and add the washing to the filtered sample.
- Oil and grease concentration of the extract can be determined using either the infrared spectrophotometry or the gravimetric method.

- Infrared spectrophotometry method
  - Quantitatively transfer the sediment extract to a convenient size volumetric flask and dilute to volume with Freon.
  - Prepare calibration standards using the reference oil.
  - Transfer required amounts of the reference material into 100-mL volumetric flasks using microliter pipettes. Dilute to volume with Freon.
  - The most appropriate pathlength for the quartz cells to be used in the spectrophotometric determination is determined by the expected sample concentration. The following information is presented as a guide for selecting cell length:

Pathlength, cm	Expected Range, mg
1	4 - 40
5	0.5 - 8
10	0.1 - 4

  Based on observed ranges of oil and grease in sediments, it may be necessary to dilute the sample extracts to the working ranges indicated above.
  - Scan the standards and samples from 3,200 to 2,700 cm^{-1} using a recording infrared spectrophotometer. Freon should be used in the reference beam of a dual beam instrument or to zero a single beam instrument. The absorbance of the 2930-cm^{-1} peak should be used to construct a standard curve.
  - Prepare a standard curve by plotting measured absorbance vs. oil and grease concentration of the standards. Compare the absorbance of the Freon extract to the standard curve to determine the oil and grease concentration.
  - Calculate oil and grease concentration as follows:

$$\text{Concentration (mg/kg dry weight)} = \frac{(X)(V)(1{,}000)}{(g)\ (\%\ S)}$$

Where:

X = concentration of oil and grease in the Freon extract, mg/L
V = volume of Freon extract, L
g = wet weight of sediment extracted, g
% S = percent total solids in the sediment sample (expressed as a decimal fraction).

- Gravimetric method
  - Quantitatively transfer the sediment extract to a tared distilling flask. Rinse the extract container with Freon and add to the distilling flask.
  - Distill the Freon from the extraction flasks using a water bath at 70° C.
  - After the solvent has been evaporated, place the flask on a warm steam bath for 15 min and draw air through the flask by means of an applied vacuum for the final 1 min.
  - Cool the sample in a desiccator for 30 min.
  - Weigh the cooled sample to the nearest 0.1 mg.
  - Calculate oil and grease concentration as follows:

$$\text{Concentration (mg/kg dry weight)} = \frac{(A-B)(1000)}{(g)(\%S)}$$

Where:

A = weight (mg) of tared flask and oil and grease residue
B = weight (mg) of tared flask
g = wet weight (g) of sediment extracted
%S = percent total solids in the sediment sample (expressed as a decimal fraction)
1000 = factor for converting mg/g to mg/kg.

QA/QC Procedures

Because the results of an oil and grease analysis are extremely sensitive to the methods used, comparable results can be obtained only by strict adherence to all methodological details.

It is critical that each sample be thoroughly homogenized in the laboratory before a subsample is taken for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

It is recommended that triplicate analyses be conducted on one of every 20 samples, or on one sample per batch if less than 20 samples are analyzed. Also, a method blank should be analyzed for each batch of samples extracted.

DATA REPORTING REQUIREMENTS

Oil and grease concentrations should be reported as mg/kg dry weight to no more than three significant figures. The laboratory should report the results of all samples (including QA replicates and method blanks) and should note any problems that may have influenced sample quality. The laboratory should also provide a description of the calibration procedures and standards used to determine oil and grease concentrations by infrared spectrophotometry.

TOTAL SULFIDES

USE AND LIMITATIONS

Total sulfides represent the amount of acid-soluble H_2S, HS^-, and S^{2-} in a sample. Sulfides are measured because they may be toxic and because they may create unaesthetic conditions. This method cannot be used if a measure of only water-soluble sulfides is desired. A measure of water-soluble sulfides might be desired if an estimate of biologically available sulfides is needed.

Sulfides are difficult to sample because some may be lost through volatilization and/or gas stripping and some may be lost through oxidation by dissolved oxygen.

FIELD PROCEDURES

Collection

Samples can be collected in glass or plastic containers. A minimum sample size of 50 g is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

Processing

Samples for total sulfides should be preserved in the field by adding 2 N zinc acetate solution (approximately 5 mL per 30 g of sediment) and swirling the mixture. Samples should be stored in the dark at 4° C and analyzed as soon as possible. It is critical that air contact with samples be minimized and samples be kept moist, to minimize oxidation. Although U.S. EPA has not established a recommended maximum holding time for sulfides in sediments, a maximum holding time of 7 days would be consistent with the holding time recommended for sulfides in preserved water samples.

LABORATORY PROCEDURES

Analytical Procedures

- Equipment
  - Distillation apparatus, all glass
    For large samples, a suitable assembly consists of a 1-L pyrex distilling flask with Graham condenser as used for the analysis of phenols. A section of glass tubing should be

connected to the tip of the condenser so that it reaches the bottom of the collection tube.

- Distillate collection tubes
  Short-form Nessler tubes, graduated at 50 and 100 mL.
- Spectrophotometer
  For use at 650 nm and providing a light path of 1 in or greater.
- Nitrogen, water-pumped
- Zinc acetate, 2 N
  Dissolve 220 g of $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ in distilled water and dilute to 1 L.
- Zinc acetate, 0.2 N
  To 100 mL of 2 N $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ add several drops of acetic acid and dilute to 1 L.
- Sulfuric acid solution, 1:1
  Carefully add 500 mL of concentrated H_2SO_4 to 500 mL of distilled water in a 1-L flask. Mix continuously and cool under running water while combining reagents. Cool solution before using.
- Dilute sulfuric acid solution, approximately 0.1 N
  Dilute 5 mL of 1:1 H_2SO_4 to 1 L with distilled water.
- Stock amine solution
  Dissolve 2.7 g of N, N-dimethyl-p-phenylenediamine sulfate and dilute to 100 mL with 1:1 H_2SO_4 solution. This solution is stable for approximately 1 wk.
- Working amine solution
  Dilute 2 mL of stock amine solution to 100 mL with 1:1 H_2SO_4 solution. Prepare fresh daily.
- Ferric chloride solution
  Dissolve 100 g of $FeCl_3 \cdot 6H_2O$ in hot distilled water and dilute to 100 mL. Cool before use.
- Standard potassium biniodate solution, 0.025 N
  Accurately weigh out 0.8124 g $KH(IO_3)_2$ and dissolve in distilled water. Dilute to 1 L.
- Standard sodium thiosulfate titrant, 0.025 N
  Dissolve 6.205 g $Na_2S_2O_3 \cdot 5H_2O$ in distilled water and dilute to 1 L. Preserve with 5 mL chloroform. Standardize against standard potassium biniodate using starch as an indicator.
- Potassium iodide solution
  Dissolve 5 g of KI in distilled water and dilute to 100 mL.
- Treated hydrochloric acid
  Place one or two strips of aluminum in a small beaker of concentrated HCl. Following the subsequent reaction, the acid is poured off and is ready to use.
- Oxygen-free dilution water
  Pass nitrogen gas through a sufficient quantity of distilled water for dilution requirements. A minimum of 10 min is required to displace oxygen in the water.
- Sodium sulfide, reagent, crystal.

- Standards preparation
  - Prepare 0.01 N sulfide solution as follows: weigh approximately 1.2 g of large crystal $Na_2S \cdot 9H_2O$. Wash the crystals several times with distilled water. Discard the washings and add the washed crystals to 975 mL of nitrogen-saturated distilled water. Dilute to 1 L.
  - Pipet 20 mL of stock sulfide solution into 100 mL of oxygen-free water. Add 5 mL of KI solution, 20 mL of 0.025 N $KH(IO_3)_2$ solution, and 10 mL of 0.1 N H_2SO_4. Titrate with 0.025 N $Na_2S_2O_3$ solution using starch as an endpoint indicator. Carry a blank through the procedure and calculate the amount of reacted iodine from the difference between the blank and standard titrations. Because 1 mL of 0.025 N KH $(IO_3)_2$ is equivalent to 0.400 mg of sulfide ion, calculate the sulfide concentration in the stock solution.
  - Calculate the volume of stock solution that contains 0.2 mg sulfide and add this amount to 900 mL of oxygen-free water. Dilute to 1 L. This is the working standard containing 2 ug S/mL. Sulfide solutions are extremely unstable and must be prepared fresh and used immediately. Stability is increased by using nitrogen-saturated water for dilution.
  - Prepare a standard curve by dilution of the working sulfide solution. Pipet 20 mL 0.2 N $Zn(C_2H_3O_2)_2$ into a series of 50-mL Nessler tubes. Add the required amounts of sulfide solution to each Nessler tube, taking care to pipet the solution below the $Zn(C_2H_3O_2)_2$ level. Dilute to 50 mL with oxygen-free water.
  - Equilibrate the temperature of the standards to 23-25° C using a water bath while the colorimetric reagents are added. Add 2 mL dilute amine-sulfuric acid solution to the standard, mix, and add 0.25 mL (5 drops) $FeCl_3$ solution. Mix the solution and allow 10 min for color development. Measure the absorbance at 650 nm.

- Sample preparation
  - Allow samples to warm to room temperature.
  - Homogenize each sample mechanically, incorporating any overlying water.
  - Remove a representative aliquot (approximately 25 g) and analyze for total solids content.
  - Remove a representative aliquot for total sulfides analysis. The aliquot should not contain more than 50 ug of sulfide.
  - Weigh the aliquot to the nearest 0.1 mg.

- Distillation
  - Set up the distillation apparatus. The transfer tube from the condenser should reach the bottom of the distillated collection tube. The condenser should be attached so that it can be easily moved up or down when diluting the distillate or adding reagents.
  - Pipet 20 mL of 0.2 N $Zn(C_2H_3O_2)_2$ into a 100-mL Nessler tube and lower the condenser so that the transfer tubing reaches below the

level of the liquid. Attach a distilling flask and pass nitrogen gas through the system for at least 10 min.

- Transfer the sample aliquot to the distillation flask. Bubble nitrogen gas through the sample to remove any oxygen dissolved in the sample. A small amount of sulfide may be driven over by the gas, so be sure that the only exit is through the zinc acetate solution in the collecting tube.
- Discontinue nitrogen evolution and add rapidly several boiling stones, two drops of methyl orange indicator, and enough treated HCl to change the color from orange to red. Stopper as quickly as possible and heat slowly. The slower the heating rate, the greater the contact time between the evolved H_2S and $Zn(C_2H_3O_2)_2$ and the less chance of sulfide loss.
- Distill the solution until approximately 20 mL of distillate has been collected (roughly 5-8 min after the solution commences to boil). Turn off heat and remove the stopper in the distillation flask to keep the distillate from being sucked back up the condenser. Raise the transfer tube above the 50-mL mark on the collection container and dilute the solution to 50 mL.
- Place the distillates in a water bath at 23-25° C. Add 2 mL dilute amine solution and mix. Add 0.25 mL (5 drops) $FeCl_3$ solution and mix. Allow 10 min for color development and measure sample absorbance at 650 nm.

- Calculations
  - Prepare a standard curve by plotting absorbance of the standards vs. sulfide concentration. Ensure that the standards cover the range of concentrations expected in the samples. Determine the sulfide concentration of the sample distillate by comparing sample absorbance with the standard curve.
  - Calculate total sulfides concentration as follows:

$$\text{Concentration (mg/kg dry weight)} = \frac{(C)(0.05)(1000)}{(g)(\%S)}$$

Where:

C = sulfide concentration in distillate, mg/L
0.05 = sample volume of distillate, L (as written)
g = wet weight of sediment aliquot, g
%S = percent solids of sediment as a decimal fraction.

QA/QC Procedures

It is critical that each sample be thoroughly homogenized in the laboratory before a subsample is taken for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

It is recommended that triplicate analyses be conducted on one of every 20 samples, or on one sample per batch if less than 20 samples are analyzed. Fresh standards should be used to calculate a calibration curve for each batch of samples. The analytical balance should be inspected daily and calibrated at least once per week.

DATA REPORTING REQUIREMENTS

Total sulfides should be reported as mg/kg of sediment dry weight to the nearest 0.1 unit. The laboratory should report the results of all samples (including QA replicates) and should note any problems that may have influenced sample quality. The laboratory should also describe the calibration curve used to determine total sulfide concentrations.

TOTAL NITROGEN

If the elemental analyzer used to measure total organic carbon can also measure total nitrogen, it is recommended that the latter variable be measured simultaneously with TOC. Total nitrogen values in sediments generally are used to compare carbon-to-nitrogen ratios. A separate total nitrogen analysis using a technique other than the elemental analyzer method is not considered equivalent for calculating carbon-to-nitrogen ratios.

BIOCHEMICAL OXYGEN DEMAND (BOD)

USE AND LIMITATIONS

Biochemical oxygen demand is a measure of the dissolved oxygen consumed by microbial organisms while assimilating and oxidizing the organic matter in a sample. This test is used to estimate the amount of organic matter that is available to organisms, in contrast to other tests used to estimate the total amount of organic matter (e.g., total volatile solids, total organic carbon, chemical oxygen demand).

In addition to oxygen used for degrading organic matter, biochemical oxygen demand may also include oxygen used to oxidize inorganic material (e.g., sulfide, ferrous iron) and reduced forms of nitrogen.

The method described by Plumb (1981) involves the use of freshwater bacteria as the seed and buffered distilled water as the dilution water. An alternative procedure for marine sediments is to use marine bacteria as the seed and filtered, oxygenated seawater as the dilution water. If seawater is used, it does not need to be buffered.

FIELD PROCEDURES

Collection

Samples can be collected in glass or plastic containers. A minimum sample size of 50 g is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

Processing

Samples should be stored at 4° C, and can be held for up to 7 days under that condition. Samples should be kept field-moist and air contact should be prevented to minimize oxidation.

LABORATORY PROCEDURES

Analytical Procedures

- Equipment
  - Incubator
    - Thermostatically controlled at 20° ± 1° C.
    - All light should be excluded to prevent the photosynthetic production of dissolved oxygen by algae in the sample.

- Incubation bottles
  300-mL capacity, with ground glass stoppers.
- Phosphate buffer solution
  Dissolve the following in distilled water: 8.5 g potassium dihydrogen phosphate, KH_2PO_4; 21.75 g dipotassium hydrogen phosphate, K_2HPO_4; 33.4 g disodium hydrogen phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$; and 1.7 g ammonium chloride, NH_4Cl. Dilute to 1 L. The pH of this buffer should be 7.2 without further adjustment. If dilution water is to be stored in the incubator, the phosphate buffer should be added just prior to using the dilution water.
- Magnesium sulfate solution
  Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 L.
- Calcium chloride solution
  Dissolve 27.5 g anhydrous $CaCl_2$ in distilled water and dilute to 1 L.
- Ferric chloride solution
  Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 L.
- Dilution water
  Store distilled water in cotton-plugged bottles for a sufficient length of time to become saturated with dissolved oxygen. The water should be aerated by shaking a partially filled bottle or using a supply of clean compressed air. The distilled water used should be as near as possible to 20° C and of high purity. Place the desired volume of distilled water in a suitable bottle and add 1 mL each of phosphate buffer, magnesium sulfate, calcium chloride, and ferric chloride for each liter of water.
- Seeding material
  Satisfactory seed may sometimes be obtained by using the supernatant liquor from domestic sewage that has been stored at 20° C for 24-36 h. Use the seed that has been found by practical experience to be the most satisfactory for the particular material under study. Only past experience can determine the amount of seed to be added per liter but the amount should give an oxygen depletion of approximately 2 mg/L. The amount of seed required may vary with the source of the seed. Seeded dilution water should be used the same day it is prepared.

- <u>Standards preparation</u>
  - Prepare a stock BOD standard solution by dissolving 0.150 g reagent grade glucose and 0.150 g reagent grade glutamic acid in 1 L of distilled water. The solids should be dried for 1 h at 103° C prior to weighing.

- Sample preparation
  - Allow samples to warm to room temperature.
  - Homogenize each sample mechanically, incorporating any overlying water.
  - Remove a representative aliquot (approximately 25 g) and analyze for total solids content.
  - Remove a representative aliquot (approximately 5 g) and weigh it to the nearest 0.1 mg.
  - Transfer the weighed aliquot to a BOD bottle for analysis.

- Analytical procedures
  - Fill each BOD bottle with dilution water and place the samples in the incubator. Ensure that air bubbles are not trapped in the BOD bottles. Prepare a blank consisting of dilution water in a separate BOD bottle. Make sure that there is a water seal in the neck of each sample bottle and blank when placed in the incubator. Replenish the water seals on all bottles each morning.
  - Determine the initial dissolved oxygen concentration of each sample and blank using the Winkler titration method or a dissolved oxygen probe. This can best be accomplished by directly measuring the dissolved oxygen concentration in the dilution water. This method is recommended because sediment may cause a rapid consumption of oxygen, making it difficult to obtain a stable initial dissolved oxygen reading. If a probe is used for oxygen measurement, the same sample can be used for immediate dissolved oxygen demand and biochemical oxygen demand.
  - Incubate samples and blanks for 5 days at 20 $\pm$ 1° C. Determine residual dissolved oxygen concentrations in the incubated samples using the analytical method of choice. The most reliable BOD determinations will occur in those samples with a residual dissolved oxygen concentration of at least 2 mg/L and a dissolved oxygen depletion of at least 2 mg/L.
  - It is recommended that the dilution water be incubated as a check on its quality. To do this, fill two BOD bottles with unseeded dilution water. Stopper one bottle, fill the water seal, and place in the incubator at 20 $\pm$ 1° C for 5 days. Analyze the second sample to determine initial dissolved oxygen concentration. Following the 5-day period, determine dissolved oxygen in the incubated sample. The oxygen depletion should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L. If these values are exceeded, the quality of the dilution water or the treatment of samples (e.g., filling of water seals) should be considered suspect.
  - Prepare a working BOD standard solution by diluting 20 mL of the stock solution to 1 L with seeded dilution water. Fill three BOD bottles and incubate at 20 $\pm$ 1° C for 5 days. The resulting BOD of these samples should be 218 $\pm$ 11 mg/L. Any appreciable deviation from these expected results may raise questions on the quality of the dilution water, the viability or suitability of the seed material, or the analytical technique.

- Calculations
  - Sediment BOD is calculated as follows:

$$\text{BOD (mg/kg dry weight)} = \frac{(O-F)(b)}{(g)(\%S)}$$

Where:

O = dissolved oxygen concentration at time zero, mg/L
F = dissolved oxygen concentration after 5 days, mg/L
b = volume of BOD bottle, mL
g = wet weight of sediment sample used, g
%S = percent solids in sediment sample (expressed as a decimal fraction.

QA/QC Procedures

It is critical that each sample be thoroughly homogenized in the laboratory before a subsample is taken for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

It is recommended that triplicate analyses be conducted on one of every 20 samples, or on one sample per batch if less than 20 samples are analyzed. A dilution water blank and glucose-glutamic acid standard should be analyzed at the same frequency as the triplicate analyses.

DATA REPORTING REQUIREMENTS

Biochemical oxygen demand should be reported as mg/kg dry weight, to the nearest 0.1 unit. The laboratory should report the results of all samples analyzed, including QA replicates, seeded dilution water blanks, unseeded dilution water blanks, and glucose-glutamic acid standards. The laboratory should also note any problems that may have influenced sample quality.

CHEMICAL OXYGEN DEMAND (COD)

USE AND LIMITATIONS

Chemical oxygen demand is a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant at elevated temperature and reduced pH. The test was devised as an alternative to the biochemical oxygen demand test for estimating organic matter. For samples from a specific source, chemical oxygen demand can be related empirically to biochemical oxygen demand, total organic carbon, or total volatile solids and then used for monitoring after a relationship has been established.

Major limitations of the chemical oxygen demand test are that it is not specific for organic matter and that correlations with other measures of organic carbon are not always found. Inorganic substances such as Fe^{2+}, Mn^{2+}, and S^{2-} can increase the consumption of oxidizing agent during the test. Plumb (1981) recommends that chemical oxygen demand not be equated with organic matter in sediments.

FIELD PROCEDURES

Collection

Samples can be collected in glass or plastic containers. A minimum sample size of 50 g is recommended. If unrepresentative material is to be removed from the sample, it should be removed in the field under the supervision of the chief scientist and noted on the field log sheet.

Processing

Samples should be stored at 4° C and can be held under that condition for 7 days. Samples must be kept field-moist and free from air contact during storage to prevent air oxidation.

LABORATORY PROCEDURES

Analytical Procedures

- Equipment
  - Reflux apparatus
    Consisting of 250- or 500-mL Erlenmeyer flasks with ground glass 24/40 neck[1] and 300-mm jacket Liebig, West, or equivalent condensers[2] with 24/40 ground glass joint.
  - Hot plate
    Having sufficient power to produce 1.4 W/cm^2 (9 W/in^2) of heating surface, or equivalent, to ensure adequate refluxing of the sample.
  - Standard potassium dichromate solution, 0.250 N
    Dissolve 12.259 g $K_2Cr_2O_7$ primary standard grade, previously dried at 103° C for 2 h, in distilled water and dilute to 1 L. The addition of 0.12 g/L sulphamic acid will eliminate interference due to nitrites in the sample at concentrations up to 6 mg/L.
  - Sulfuric acid reagent
    Concentrated H_2SO_4 containing 22 g silver sulfate, Ag_2SO_4, per 9-lb bottle. Allow 1 or 2 days for dissolution.
  - Standard ferrous ammonium sulfate titrant, 0.25 N
    Dissolve 98 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Carefully add 20 mL concentrated H_2SO_4, cool, and dilute to 1 L. This solution must be standardized against $K_2Cr_2O_7$ daily. To standardize the ferrous ammonium sulfate, dilute 10 mL standard potassium dichromate solution to approximately 100 mL. Carefully add 30 mL concentrated H_2SO_4 and allow to cool. Titrate with ferrous ammonium titrant, using 2-3 drops of ferroin indicator.

$$\text{Normality} = \frac{(\text{mL } K_2Cr_2O_7)(0.25)}{[\text{mL } Fe(NH_4)_2(SO_4)_2]}$$

  - Ferroin indicator
    Dissolve 1.485 g 1,10-phenantroline monohydrate and 0.695 g ferrous sulfate, $FeSO_4 \cdot 7H_2O$, in water and dilute to 100 mL. Alternatively, a commercially prepared indicator can be purchased.
  - Silver sulfate
    Ag_2SO_4, reagent powder.
  - Mercuric sulfate
    $HgSO_4$, analytical-grade crystals.

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[1]Corning 5000 or equivalent.

[2]Corning 2360, 91548, or equivalent.

- Sample preparation
  - Allow samples to warm to room temperature.
  - Homogenize each sample mechanically, incorporating any overlying water.
  - Remove a representative aliquot (approximately 25 g) and analyze for total solids content.
  - Remove a representative aliquot (approximately 2 g) and weigh it to the nearest 0.1 mg.
  - Transfer the weighed aliquot to a reflux flask for COD analysis. Wash the sediment into the flask with a minimum amount of distilled water (i.e., <25 mL).

- Analytical procedures
  - Place several boiling stones or glass beads and 1.0 g $HgSO_4$ in the reflux flask with the sample.
  - Add 25 mL 0.25 N $K_2CR_2O_7$ to the flask and mix thoroughly.
  - Slowly, and with constant mixing, add 75 mL of sulfuric acid-silver sulfate solution. Ensure that the mixture is well mixed to avoid localized superheating.
  - Attach the sample flask to a condenser and reflux for 2 h. If the added dichromate dissipates during reflux, either 1) repeat, using a smaller sample size, or 2) carefully add additional 0.25 N $K_2Cr_2O_7$ through the condenser. Be sure to record any added dichromate.
  - Allow the sample to cool and rinse the condenser with 40-50 mL distilled water.
  - Add an additional 50 mL of distilled water to the sample and allow to cool to room temperature.
  - Add 3-5 drops of ferroin indicator and titrate with 0.25 N $Fe(NH_4)_2(SO_4)_2$ to a sharp color change (blue-green to reddish-brown).
  - For a blank, reflux 25 mL of distilled water, 25 mL of 0.25 N $K_2Cr_2O_7$, 1 g $HgSO_4$, several glass beads or boiling stones, and 75 mL of sulfuric acid-silver sulfate solution for 2 h. Cool, add 3-5 drops of ferrion indicator, treat as a sample and titrate with 0.25 N $Fe(NH_4)_2(SO_4)_2$.

- Calculations
  - Sediment COD is determined as follows:

$$\text{COD (mg/kg dry weight)} = \frac{(A-B)(N)(8{,}000)}{(g)\ (\%S)}$$

Where:

A = volume of 0.25 N $Fe(NH_4)_2(SO_4)_2$ for blank titration, mL
B = volume of 0.25 N $Fe(NH_4)_2(SO_4)_2$ for sample titration, mL
N = normality of 0.25 N $Fe(NH_4)_2(SO_4)_2$ used for titration, eq/L
8,000 = equivalent weight of oxygen, mg/eq

g = wet weight of sample, g
%S = percent solids in sediment sample (expressed as a decimal fraction.

QA/QC Procedures

It is critical that each sample be thoroughly homogenized in the laboratory before a subsample is taken for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field.

It is recommended that triplicate analyses be conducted on one of every 20 samples, or on one sample per batch if less than 20 samples are analyzed.

DATA REPORTING REQUIREMENTS

Chemical oxygen demand should be reported as mg/kg of sediment dry weight to the nearest 0.1 unit. The laboratory should report the results of all samples analyzed (including QA replicates and method blanks) and should note any problems that may have influenced data quality. The laboratory should also report the results of the standard test.

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METALS

FINAL REPORT
TC-3090-04

Puget Sound Estuary Program

RECOMMENDED PROTOCOLS FOR MEASURING METALS IN PUGET SOUND WATER, SEDIMENT, AND TISSUE SAMPLES

Prepared by:
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Prepared for:
RESOURCE PLANNING ASSOCIATES

for:
PUGET SOUND DREDGED DISPOSAL
ANALYSIS (PSDDA)

Monitored by:
U.S. Army Corps of Engineers
Seattle District
Seattle, WA

August, 1986

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This chapter was prepared by Tetra Tech, Inc., under the direction of Mr. Robert Barrick, for Resource Planning Associates (RPA) for the Puget Sound Dredged Disposal Analysis (PSDDA) monitored by the Seattle District, U.S. Army Corps of Engineers (COE) in partial fulfillment of Contract No. DACW67-85-D-0029. Dr. David Kendall of U.S. COE was the Technical Coordinator. The primary author of this chapter was Dr. Charles Lytle of Tetra Tech, Inc.

INTRODUCTION

GENERAL

This chapter presents recommended protocols for measuring metals in water, sediment, and tissue samples from Puget Sound. The list of metals considered and their limits of detection (LODs) are summarized in Appendix A. The main objectives of this report are to:

- Elucidate acceptable method options for each metal meeting the minimum LODs specified for water, sediment, and biological samples from Puget Sound
- Prioritize all method options for each metal and make recommendations on the best method for each appropriate matrix.

The recommendations presented in this chapter are based on the results of a workshop and detailed reviews by representatives from most organizations that fund or conduct environmental research in Puget Sound (Table 1). The purpose of developing these recommended protocols is to encourage all Puget Sound investigators conducting monitoring programs, baseline surveys, and intensive investigations to use standardized methods whenever possible. If this goal is achieved, most data collected in Puget Sound should be directly comparable and thereby capable of being integrated into a sound-wide database. Such a database is necessary for developing and maintaining a comprehensive water quality management program for Puget Sound.

Although the following protocols are recommended for most studies conducted in Puget Sound, departures from these methods may be necessary to meet the special requirements of individual projects. If such departures are made, however, the funding agency or investigator should be aware that the resulting data may not be comparable with most other data of that kind. In some instances, data collected using different methods may be compared if the methods are intercalibrated adequately.

PRECAUTIONS AND LIMITATIONS

Although well-established procedures for proper sample handling and analysis are essential for collecting data of high quality, standard protocols do not exist for all the required methods. Approved methods exist for the analysis of metals in water (e.g., U.S. EPA 1979a, ASTM 1983), but only interim procedures have been proposed for sediment and tissue samples (U.S. EPA 1977, Plumb 1981). New considerations may need to be addressed and additional guidelines provided to maximize the quality and efficiency of the procedures.

TABLE 1. CONTRIBUTORS TO THE METALS PROTOCOLS

Roy Araki[a]	Washington Dept. of Ecology
Bob Barrick[a,b]	Tetra Tech
Robert Clark[a]	NOAA/NMFS
Eric Crecelius[a]	Battelle Northwest
Ray Dalseg[a]	Metro
Bob Dexter[a]	EVS Consultants
Mark Fugiel[a]	Am Test
Michael Higgins[a]	Analytical Technologies
Roger Kadeg[a]	Envirosphere
David Kendall[a]	U.S. COE
Mary Beth Lanza[a]	Laucks Testing Labs
Chuck Lytle[a]	Tetra Tech
Pamela Navaja[a]	Washington DSHS
Matt Schultz[a]	Envirosphere
Mike Shelton	Weyerhaeuser Co.
Jeff Stern[a]	Tetra Tech
Steve Twiss[a]	Washington Dept. of Ecology

[a] Attended the workshop held on January 17, 1986.

[b] Workshop moderator.

The instrumental methods proposed are prone to chemical and matrix interferences that can either suppress or enhance the analyte signal (Skoog 1985). Special attention should be given when they are likely to be present as noted in the individual protocols. If any such interferences are suspected, it is extremely important that their effect be determined and corrective action taken.

Because of the low limits of detection proposed for many environmental analyses, clean field and laboratory procedures are especially important. Sample contamination can occur during any stage of collection, handling, storage, or analysis. Potential contaminant sources must be known and steps taken to minimize or eliminate them (Murphy 1976).

SAMPLE COLLECTION

PRECOLLECTION

Container Materials

The best containers for the collection and/or storage of trace metal samples are made of quartz or tetrafluoroethylene (TFE). Because these containers are expensive, the preferred container material is either polypropylene or linear polyethylene, with a polyethylene cap. Borosilicate glass may be used, but lids must not have aluminum or cardboard liners (Greenberg et al. 1985).

For tissue samples, scalpels should be made of corrosion-resistant stainless steel, while tweezers and cutting surfaces should be plastic or TFE (Tetra Tech 1986a). Stainless steel homogenizer blades should be replaced with tantalum.

The recommended container materials, sample sizes, preservation techniques, and storage lifetimes for all the metals of concern in water, sediment, and tissue are summarized in Table 2.

Cleaning Methods

Prior to use, containers and any glass or plastic parts associated with the sampling equipment should be thoroughly cleaned with a detergent solution, rinsed with tap water, soaked 24 h at 70° C in an acid solution of 1:1 HNO_3 or 1:1 HCl, and then rinsed with metal-free water. Do not use chromic acid for any cleaning purpose. For stainless steel parts, omit the acid soaking step (Greenberg et al. 1985).

SOURCES OF CONTAMINATION

In the field, sources of contamination include sampling gear, lubricants and oils, engine exhaust, airborne dust, and ice used for cooling samples. During sample handling and analysis, contamination sources include exposure to airborne dust, insufficiently clean sample containers, contact with inappropriate materials (e.g., rubber o-rings, aluminum-lined caps, steel containers), contaminated reagents, and carryover in testing instruments due to insufficient cleaning or flushing between samples.

Exposure to airborne dust can be minimized by using capped containers and by keeping physical sample handling to a minimum. Use of recommended container materials and cleaning methods should minimize contamination from storage containers. Reagents should be ACS Reagent Grade or better and should never be returned to their stock containers once removed. Carryover can be avoided by carefully following instrument manufacturers' instruc-

TABLE 2. SAMPLE PRESERVATION AND STORAGE PARAMETERS

Analyte	Matrix	Sample Container[b]	Sample Size	Storage Preservative	Storage Lifetime
Total or dissolved metals (except Hg)[a]	H_2O	P,G,TFE[g]	1 L	HNO_3 pH <2	6 mo
Total or dissolved Hg[a]	H_2O	G,TFE	250 mL	HNO_3 pH <2	28 days
Particulate Metals	H_2O	P,G	1 gal	i	i
All metals	Sediment	P,G	50 gc	Freeze	6 mo[d]
Elutriate Study[e]	H_2O Sediment	P,G P,G	12 L 3 L	4° C 4° C	-- --
Fractionation Study[e]	Sediment	P,G	1 L	4° C	--
All metals (except Hg)	Tissue	P,G	5 gc,h	Freeze[f]	6 mo[d]
Hg	Tissue	P,G	0.2 gc,h	Freeze[f]	28 days[d]

a U.S. EPA 1982.

b P = linear polyethylene, G = borosilicate glass, TFE = tetrafluoroethylene.

c Wet weight.

d Suggested. No EPA criteria exist. Hg holding time 28 days.

e Plumb 1981. Storage time "as short as possible," analyses to be completed "within 1 wk of sample collection."

f Post-dissection.

g If aliquot for Hg taken from this 1 L sample, cannot use linear polyethylene.

h Weight is a minimum for one sample. Studies using specific organs may require more.

i Samples should be filtered as soon as possible and always within 24 h. Workshop attendees recommend that filtering be done shipboard rather than in lab on shore.

tions. Sources of contamination in the analytical laboratory and on shipboard have been thoroughly reviewed by Mart (1979a,b).

WATER COLUMN SAMPLES

Water column samples frequently are collected using water bottles. Water bottle samplers are relatively simple devices that generally consist of some type of cylindrical tube with stoppers at each end and a closing device that is activated by a messenger or an electrical signal. The most commonly used samplers of this description are the Kemmerer, Van Dorn, Niskin, and Nansen samplers. Each device samples a discrete parcel of water at any designated depth. Frequently, multiple water samplers are fixed on a rosette frame so that several depths can be sampled during one cast or replicate samples can be taken at the same depth.

Prior to deployment, the stoppers of water bottle samplers are cocked open on the sampling vessel. At this step, it is critical that the interior of the sampler and stoppers remain free from contamination. All members of the sampling team should therefore avoid touching the insides of the sampler and stoppers, and all samplers and stoppers must be rinsed thoroughly.

After cocking, the sampler is lowered to a designated depth. The sampler must be open at both ends so that water is not trapped within the device as it is being lowered through the water column. Once the sampler reaches the desired depth, it should be allowed to equilibrate with ambient conditions for 2-3 min before being closed.

After equilibration, the closing device can be activated by messenger or electrical signal, and the sampler can be retrieved. It is recommended that at least two samplers be used simultaneously for each depth. A second sampler will provide a backup to the primary sampler in case the latter device misfires or won't trigger. This will eliminate the need for an additional cast. A second sampler will also supplement the primary sampler if the volume collected by the latter device is too small for all required subsampling and rinsing. To ensure that all subsamples at a particular depth are collected from the same water parcel, it is essential that they all be taken from a single cast. Multiple casts using a single water sampler will not meet this objective. Sample water must therefore be used conservatively after collection.

Once the water sampler is brought on board the sampling vessel, the stoppers should be checked immediately for complete seals. If a stopper is not properly sealed, water from the sampled depth may have leaked out upon retrieval and been replaced by water from shallower depths. Because this kind of contamination can bias results, the entire water sample should be rejected.

PARTICULATE SAMPLES

This section provides guidance on the collection of the particulate fraction of water samples from in-plant process streams, effluents, and receiving waters. All devices capable of separating particulate material from water samples may contribute contaminants to the sample taken for analysis. The level of this "blank" contamination must be determined in advance and reduced when possible.

There are several techniques for obtaining the particulate fraction of water samples, including different filtration procedures, settling/centrifugation, centrifugation, and continuous-flow centrifugation. The latter technique may not be appropriate for metals because the equipment required contains a number of metallic parts. A backflush-filtration technique has also been described in an intercomparison study (Horowitz 1986), but is not appropriate for chemical analyses because of selective losses of the filtered material prior to analysis.

Filtration may be prefereable to other techniques for many analyses because it requires less expensive equipment (i.e., a high speed centrifuge is not required), and provides a sample that is suitable for direct chemical analysis (i.e., a residue on a filter that can be extracted or digested). The amount of particulate material that may be collected on a filter (e.g., typically <1 g) may limit this technique for low-level analyses, including metals. Centrifugation techniques can yield comparable results and may be used to collect much larger amounts of particulate material than by filtration (e.g., several grams), but require careful and complete transfer of the sample from the centrifuge prior to analysis.

Horowitz (1986) describes a centrifugation technique (fixed angle-head, or swing-bucket rotor; not a continuous-flow system) for metals analysis that he recommends when large-scale studies are undertaken (e.g., 2,000-3,000 samplers per year). Bates et al. (1983) describe a system using continuous-flow centrifugation (Sorvall Model SS-3 or RC-5 high-speed centrifuge) for collection of particulate material for organic analyses (specifically hydrocarbons). One interpretation of the results of this study that may have implications for metals analyses was the possibility that continuous-flow centrifugation may not recover fine-grained particles in the 1-2um range as efficiently as filtration. Although both techniques recovered between 70 and 90 percent of the particles in this size range, these very small particles may have a higher loading of metals than larger particles.

Sample Compositing and Preservation

Ideally, samples of in-plant process streams or effluents should be collected as a flow-proportionate composite of at least 5 days of mechanical interval sampling to yield a time-integrated measurement. If flow-proportionated samples cannot be collected (i.e., aliquots collected in proportion to the changing flow rate of the stream), instantaneous grab samples can

be substituted, but should still be composited over several days collection whenever possible. It is usually unreasonable to composite receiving water samples over such periods of time in a routine sampling program, and it may be unreasonable to store composited process stream or effluent samples over several days when total volumes greater than approximately 20 L (i.e., 5 gal) are required.

Samples for metals analysis should be stored under refrigeration in 1-gal polyethylene cubitainers. Until use, the cubitainers should be stored (collapsed) in plastic bags. The cubitainers should be carefully rinsed with several aliquots of the sample prior to filling to remove any dust particles. An acid pre-wash step is not considered necessary for the cubitainers.

Filtration of the entire sample should be conducted at one time, and this process may take from one to several hours to complete. A potential preservation technique is addition of mercuric chloride (for organic analyses only), or a 2 percent sodium azide solution. The latter preservative has worked well with sediment trap samples collected by NOAA, but requires access to a fume hood for sample processing which is a distinct limitation. Suspended solids samples cannot be preserved with 100 percent effectiveness (Greenberg et al. 1985). Hence, preserved sample bottles should be stored under refrigeration (2°C) prior to filtration to minimize decomposition of solids. No samples should be held for longer than 7 days.

Sample Volumes

A 4-L composited sample (i.e., 1-gal) is recommended for the analysis of metals in particulate samples. The volume required for analysis is a function of the concentration of particulate material in the stream and the concentration of metals in the particulate material. Horowitz (1986) recommends the collection of 100 mg of particulate material for analysis of metals, but this quantity cannot always be obtained with a 4-L sample. Larger sample volumes (e.g., 20-L) can be processed but are not considered necessary for most purposes. Smaller sample sizes (e.g., 5-30 mg) have been successfully analyzed for most metals using graphite furnace and ICP techniques and a final dilution volume of 10 mL digestate (Tetra Tech 1985).

Sample Processing

Preparation of Filters--

Polycarbonate membrane filters (e.g., Nuclepore) of 0.4-um pore size are recommended for filtration of water samples for metals analyses. The 142-mm diameter filters are required to filter the recommended minimum of approximately 4-L of sample. The membrane filters do not require any pretreatment prior to weighing, and can be taken directly from the manufacturer's container. Each filter must be carefully weighed to the nearest 0.1 mg prior to filtering. Unlike glass fiber filters, the amount of residue

collected on membrane filters can be accurately weighed. The dry weight of the filtered material is used directly to determine the concentration of metals associated with the particulate material.

Because the 142-mm filters are so light, care must be taken to prevent wrinkling when placing the filter in the filter holder. Air currents can easily disrupt the placement of the filter, and once wet the filters must be carefully moved to their final position on the holder to prevent tearing. A TFE filter holder is recommended, but a plastic filter holder may also be used.

Filtration of Samples

After the tared filter has been placed into the filter holder, a plastic squeeze bottle should be used to completely rinse the filter with 10 percent high purity HCl followed by deionized, distilled water until neutral pH has been established. A water aspirator can be used to provide suction for the rinsing. The acid rinse will leach any dust particles that may have adhered to the filters (such particles can contribute zinc and potentially other contaminants to the analysis).

Once the filter has been properly rinsed, the filter holder should be reassembled and a siphon tube attached between the filter holder and the cubitainer holding the water sample (place the cubitainer above the filter holder to provide gravity-feed). The cubitainer should be vigorously shaken prior to the filtration to dislodge particles that may have adhered to the sides. Using the water aspirator, siphon the entire water sample through the filter holder. After the water has passed through the filter, disconnect the filter holder from the aspirator while the aspirator is still running.

Carefully open the filter holder and, using plastic forceps, fold the filter in thirds (like a crepe) to protect the filtered material. Finally, with two pairs of forceps gently hold down one end of the filter while folding the filter in half from the opposite end. Place the folded filter in a polycarbonate petri dish. Seal the petri dishes, label,and store frozen until the metals analysis can be started. Prior to digestion, the filters should be dried at no more than 60°C and weighed to determine total sample weight (the original weight of the clean filter must be supplied to the laboratory). Filters dried at higher temperatures may suffer losses of the more volatile metals.

At least one filter blank should be prepared by going through the entire filtration setup procedure (i.e., including the acid-rinse step) in the absence of a water sample. Fold and place the filter blank in a polycarbonate petri dish as with other samples. At least one filter blank should consist of an untreated folded membrane filter taken directly from the manufacturer's box (to check efficiency of the rinse step).

SURFICIAL SEDIMENT SAMPLES

This section describes the protocols required to collect an acceptable subtidal surficial sediment sample for subsequent measurement of physical and chemical variables. This subject has generally been neglected in the past and sampling crews have been given relatively wide latitude in deciding how to collect samples. However, because sample collection procedures influence the results of all subsequent laboratory and data analyses, it is critical that samples be collected using acceptable and standardized techniques.

Design of Sampler

In Puget Sound, the most common sampling device for subtidal surficial sediments is the modified van Veen bottom grab. However, a variety of coring devices is also used. The primary criterion for an adequate sampler is that it consistently collect undisturbed samples to the required depth below the sediment surface without contaminating the samples. An additional criterion is that the sampler can be handled properly on board the survey vessel. An otherwise acceptable sampler may yield inadequate sediment samples if it is too large, heavy, or awkward to be handled properly.

Collection of undisturbed sediment requires that the sampler:

- Create a minimal bow wake 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.

Most modified van Veen grabs have open upper faces that are fitted with rubber flaps. Upon descent, the flaps are forced open to minimize the bow wake, whereas upon ascent, the flaps are forced closed to prevent sample winnowing. Some box corers have solid flaps that are clipped open upon descent and snap shut after the corer is triggered. Although most samplers seal adequately when purchased, the wear and tear of repeated field use eventually reduces this sealing ability. A sampler should therefore be monitored constantly for sample leakage. If unacceptable leakage occurs, the sampler should be repaired or replaced. If a sampler is to be borrowed or leased for a project, its sealing ability should be confirmed prior to sampling. Also, it is prudent to have a backup sampler on board the survey vessel in case the primary sampler begins leaking during a cruise.

The required penetration depth below the sediment surface is a function of the desired sample depth (see Penetration Depth). Generally, it is better to penetrate below the desired sample depth to minimize sample disturbance when the sampling device closes. Penetration depth of most sampling devices varies with sediment character, and generally is greatest in fine sediments and least in coarse sediments. Sampling devices generally rely upon either gravity or a piston mechanism to penetrate the sediment.

In both cases, penetration depth can be modified by adding or subtracting weight from the samplers. Thus, it is optimal to use a sampler that has a means of weight adjustment. If a sampler cannot consistently achieve the desired penetration depth, an alternate device should be used.

Once the sampler is secured on board the survey vessel, it is essential that the surface of the sample be made accessible without disturbing the sample. Generally, samplers have hinged flaps on their upper face for this purpose. The opening(s) in the upper face of the sampler should be large enough to allow easy subsampling of the sediment surface. If an opening is too small, the sample may be disturbed as the scientific crew member struggles to take a subsample.

Penetration Depth

For characterizing surficial sediments in Puget Sound, it is recommended that the upper 2 cm of the sediment column be evaluated. When collecting the upper 2 cm of sediment, it is recommended that a minimum penetration depth of 4-5 cm be achieved for each acceptable sample.

Although the 2-cm specification is arbitrary, it will ensure that:

- Relatively recent sediments are sampled
- Adequate volumes of sediments can be obtained readily for laboratory analyses
- Data from different studies can be compared validly.

Sampling depths other than 2 cm may be appropriate for specific purposes. For example, the upper 1 cm of sediment may be required to determine the age of the most recently deposited sediments. By contrast, a sample depth much greater than 2 cm may be required to evaluate the vertical profile of sediment characteristics or to determine depth-averaged characteristics prior to dredging. If a sampling depth other than 2 cm is used, comparisons with data from 2-cm deep samples may be questionable.

Operation of Sampler

The sampling device should be attached to the hydrowire using a ball-bearing swivel. The swivel will minimize the twisting forces on the sampler during deployment and ensure that proper contact is made with the bottom. For safety, the hydrowire, swivel, and all shackles should have a load capacity at least three times greater than the weight of a full sampler.

The sampler should be lowered through the water column at a controlled speed of approximately 1 ft/sec. Under no circumstances should the sampler be allowed to "free fall" to the bottom, as this may result in premature triggering, an excessive bow wake, or improper orientation upon contact with the bottom. The sampler should contact the bottom gently and only its weight or piston mechanism should be used to force it into the sediment.

After the sediment sample is taken, the sampler should be raised slowly off the bottom and then retrieved at a controlled speed of approximately 1 ft/sec. Before the sampler breaks the water surface, the survey vessel should head into the waves (if present) to minimize vessel rolling. This maneuver will minimize swinging of the sampler after it breaks the water surface. If excessive swinging occurs or if the sampler strikes the vessel during retrieval, extra attention should be paid to evaluating sample disturbance when judging sample acceptability.

The sampler should be secured immediately after it is brought on board the survey vessel. If the sampler tips or slides around before being secured, extra attention should be paid to evaluating sample disturbance.

Sample Acceptability Criteria

After the sampler is secured on deck, the sediment sample should be inspected carefully before being accepted. The following acceptability criteria should be satisfied:

- The sampler is not over-filled with sample so that the sediment surface is pressed against the top of the sampler
- Overlying water is present (indicates minimal leakage)
- The overlying water is not excessively turbid (indicates minimal sample disturbance)
- The sediment surface is relatively flat (indicates minimal disturbance or winnowing)
- The desired penetration depth is achieved (i.e., 4-5 cm for a 2-cm deep surficial sample).

If a sample does not meet all criteria, it should be rejected.

Sample Collection

After a sample is judged acceptable, the following observations should be noted on the field log sheet:

- Station location
- Depth
- Gross characteristics of the surficial sediment
  - Texture
  - Color
  - Biological structures (e.g., shells, tubes, macrophytes)
  - Presence of debris (e.g., wood chips, wood fibers, human artifacts)
  - Presence of oily sheen
  - Odor (e.g., hydrogen sulfide, oil, creosote)
- Gross characteristics of the vertical profile
  - Changes in sediment characteristics
  - Presence and depth of redox potential discontinuity (rpd) layer

- Penetration depth
- Comments related to sample quality
  - Leakage
  - Winnowing
  - Disturbance.

Before subsamples of the surficial sediments are taken, the overlying water must be removed. The preferred method of removing this water is by slowly siphoning it off near one side of the sampler. Methods such as decanting the water or slightly cracking the grab to let the water run out are not recommended, as they may result in unacceptable disturbance or loss of fine-grained surficial sediment and organic matter.

Once the overlying water has been removed, the surficial sediment can be subsampled. It is recommended that subsamples be taken using a flat scoop shaped like a coal shovel. The shoulders of the scoop should be 2 cm high. This device will allow a relatively large subsample to be taken accurately to a depth of 2 cm. Coring devices are not recommended because generally they collect small amounts of surficial sediment and therefore require repeated extractions to obtain a sufficient volume of material for analysis of conventional sediment variables. A curved scoop is not recommended because it does not sample a uniform depth. Because accurate and consistent subsampling requires practice, it is advisable that an experienced person perform this task.

When subsampling surficial sediments, unrepresentative material should be removed in the field under the supervision of the chief scientist and noted on the field log sheet. The criteria used to determine representativeness should be determined prior to sampling.

Finally, if samples are to be analyzed for trace metals or priority pollutant organic compounds, sample contamination during collection must be avoided. All sampling equipment (i.e., siphon hoses, scoops, containers) should be made of noncontaminating material and should be cleaned appropriately before use. Samples should not be touched with ungloved fingers. In addition, potential airborne contamination (e.g., stack gases, cigarette smoke) should be avoided. Detailed guidance for preventing sample contamination is given in a previous section of this chapter.

TISSUE SAMPLES

Collection

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. Care must be taken during handling to avoid these and any other possible sources of contamination. For example, during sampling the ship should be positioned such that the engine exhausts do not fall on deck. To avoid contamination from melting

ice, the samples should be wrapped in aluminum foil and placed in watertight plastic bags. The outer skin of the fish or shell of the shellfish acts as protection against metals contamination from the aluminum foil.

Sample resection and any subsampling of the organisms should be carried out in a controlled environment (e.g., dust-free room). In most cases, this requires that the organisms be transported on ice to a laboratory, rather than being resected on board the sampling vessel. It is recommended that whole organisms not be frozen prior to resection if analyses will be conducted only on selected tissues, because freezing may cause internal organs to rupture and contaminate other tissue. If organisms are eviscerated on board the survey vessel, the remaining tissue (e.g., muscle) may be wrapped as described above and frozen.

Resection is best performed under "clean room" conditions. The "clean room" should have positive pressure and filtered air. The "clean room" should also be entirely metal-free and isolated from all samples high in contaminants (e.g., hazardous waste). At a minimum, care should be taken to avoid contamination from dust, instruments, and all materials that may contact the samples. The best equipment to use for trace metal analyses is made of quartz, TFE, polypropylene, or polyethylene. Stainless steel that is resistant to corrosion may be used if necessary. Corrosion-resistant stainless steel is not magnetic, and thus can be distinguished from other stainless steels with a magnet. Stainless steel scalpels have been found not to contaminate mussel samples (Stephenson et al. 1979). However, low concentrations of heavy metals in other biological tissues (e.g., fish muscle) may be contaminated significantly by any exposure to stainless steel. Quartz utensils are ideal but expensive. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer tissue and for removing tissue for analysis. For bench liners and bottles, borosilicate glass would be preferred over plastic if trace organic analyses are to be performed on the same sample.

Resection should be conducted by or under the supervision of a competent biologist. For fish samples, special care must be taken to avoid contaminating target tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The incision "troughs" are subject to such contamination. Thus, they should not be included in the sample. In the case of muscle, a "core" of tissue is taken from within the area boarded by the incision troughs, without contacting them. Unless specifically sought as a sample, the dark muscle tissue that may exist in the vicinity of the lateral line should not be mixed with the light muscle tissue that consitutes the rest of the muscle tissue mass.

Prior to use, utensils and bottles should be thoroughly cleaned with a detergent solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. For quartz, TFE, or glass containers, use 1+1 HNO_3, 1+1 HCl, or aqua regia (3 parts conc HCl + 1 part conc HNO_3) for soaking. For plastic material, use 1+1 HNO_3 or 1+1 HCl. Reliable soaking conditions are 24 h at 70° C (Greenburg et al. 1985). Do not use chromic acid for

cleaning any materials. Acids used should be at least reagent grade. For metal parts, clean as stated for glass or plastic, except omit the acid soak step. If trace organic analyses are to be performed on the same samples, final rinsing with methylene chloride is acceptable.

Sample size requirements can vary with tissue type (e.g., liver or muscle) and detection limit requirements. In general, a minimum sample size of 6 g (wet weight) is required for the analysis of all priority pollutant metals. To allow for duplicates, spikes, and required reanalysis, a sample size of 50 g (wet weight) is recommended. Samples can be stored in glass, TFE, or high-strength polyethylene jars.

Processing

Samples should be frozen after resection and kept at -20° C. Although specific holding times have not been recommended by U.S. EPA, a maximum holding time of 6 mo (except for mercury samples, which should be held a maximum of 28 days) would be consistent with that for water samples.

When a sample is thawed, the associated liquid should be maintained as a part of the sample. This liquid will contain lipid material. To avoid loss of moisture from the sample, partially thawed samples should be homogenized. Homogenizers used to grind the tissue should have tantalum or titanium parts rather than stainless steel parts. Stainless steel blades used during homogenization have been found to be a source of nickel and chromium contamination.

ANALYTICAL METHODS

SAMPLE PREPARATION

Water: Total Metals

Freshwater samples to be analyzed by graphite furnace atomic absorption spectroscopy (GFAA) should be prepared using an HNO_3/H_2O_2 digest. Analysis by inductively-coupled plasma emission spectroscopy (ICP) or direct flame atomic absorption spectroscopy (DFAA) requires an HNO_3/HCl digest. Samples to be analyzed for mercury by cold vapor atomic absorption spectroscopy (CVAA) must have a separate, 100-mL aliquot digested using potassium permanganate and potassium persulfate. All three procedures are detailed in Exhibit D of the Contract Laboratory Program (U.S. EPA 1985), which is attached to this report as Appendix B.

Because digestion creates excessive salt concentrations that interfere with instrumental methods, saltwater samples must be extracted. Extraction with ammonium pyrrolidine dithiocarbamate/methyl isobutylketone (APDC/MIBK) is recommended and is detailed in Appendix F (Greenburg et al. 1985).

Water: Dissolved Metals

According to convention, "dissolved" substances are those contained in the filtrate passing a 0.45 um filter. Samples to be analyzed for dissolved metals must be filtered prior to preservation and subsequent digestion. However, filters are a significant source of trace element contamination. Membrane filters (e.g., polycarbonate, fluorocarbon) are significantly cleaner than paper (Murphy 1976).

It is recommended that 0.45 um polycarbonate filters without grid marks be used for the filtration of water samples and that they be cleaned by drawing through 500 mL 1:1 HNO_3 followed by 1 L metal-free water (to neutral pH). Prior to use, filters must be stored in a tightly-closed container to avoid contamination from airborne dust.

The filtrate should be acidified to pH<2 using 1:1 HNO_3. If a precipitate appears upon acidification, the sample must be digested as for total metals.

Water: Particulate Metals

Particulate substances are operationally defined as those retained on a 0.45 um filter. Polycarbonate filters should be used and cleaned as detailed in the previous section. After cleaning, the filters should be dried to constant weight at 60° C. A detailed protocol for shipboard filtration of large volumes of seawater using commercially-available equipment is given by Mart (1979b). A completely submersible, self-contained filtration apparatus, capable of filtering up to 1,000 L at a flow rate of 50-200 mL/min,

is commercially available from Seastar Instruments Ltd., Sidney, British Columbia, Canada. After sample filtration, the filters should be dried again to constant weight at 60° C. Total sample weight is then calculated by difference.

After weighing, the entire filter plus sample is digested as for sediment samples as detailed in Appendix D.

Sediment: Bulk Analysis

The two most common digestion methods employed for sediment samples are total and strong acid. Total digestion is the most severe and employs hydrofluoric acid to aid in the breakdown of silicate matrices and/or perchloric acid to aid in the decomposition of organic matter. The use of a total acid digest has been recently endorsed by the NOAA/NBS Quality Assurance Workshop (December 5-6, 1985).

Strong acid digestion employs nitric acid, some ratio of nitric and hydrochloric acids, or nitric acid and hydrogen peroxide. Strong acid digestions are not as efficient as total acid methods for the solubilization of certain metals (e.g., aluminum, chromium) that are tightly matrix-bound, but they do not require special labware or hoods. The efficiencies of various strong acid digestion methods have been reviewed by Plumb (1981). Although Plumb concludes that the literature for strong acid digests supports the use of HNO_3:HCl, the U.S. Environmental Protection Agency Contract Laboratory Program (CLP) requires the use of HNO_3:H_2O_2 (U.S. EPA 1985). The detailed procedure for the latter method is given in Exhibit D of the CLP, which is attached to this report as Appendix B.

A hydrofluoric acid/aqua regia total acid digestion was agreed upon at the January 17, 1986, PSDDA/PSEP workshop. The detailed protocol is attached to this report as Appendix D (Rantala and Loring 1975).

Sediment: Elutriate and Fractionation Studies

The elutriate test is used to estimate the types and amounts of substances likely to be released to the water column from sediments during dredging operations. Fractionation studies estimate the distribution and mobility of substances occurring in sediments. Detailed procedures for these two tests are given by Plumb (1981) and are attached to this report as Appendix C. The filtered samples from the elutriate test are treated as "dissolved metals" samples discussed above. The filtered samples from the six different "phases" of the fractionation procedure should be analyzed directly.

Tissue

Workshop participants agreed upon a nitric acid/perchloric acid digestion for tissue samples. Perchloric acid is especially useful for the dissolution of fats. The detailed procedure for this method is attached to this report as Appendix E (Tetra Tech 1986a). Equivalent methods may be used (e.g.,

U.S. EPA 1977) if accuracy and precision can be demonstrated to the level specified in the Quality Assurance/Quality Control section of this report.

INSTRUMENTAL METHODS

The instrumental methods currently available for the analysis of trace metals include electrochemical (e.g., differential pulse polarography), spectrophotometric (e.g., silver diethyldithiocarbamate), atomic absorption spectrophotometric, atomic emission spectrophotometric, x-ray fluorescence, and neutron activation.

While any analytical method might be used as long as the documented instrument or method detection limits meet the required LODs specified (Appendix A), certain instrumental techniques have drawbacks and are not recommended. For example, both electrochemical and spectrophotometric techniques are time-consuming and are not amenable to the analysis of large numbers of samples. Neutron activation requires the use of expensive nuclear equipment that is not widely distributed. Certain specialized techniques designed for the analysis of particular metal species (e.g., organotins) also require the use of research-grade instrumentation often extensively modified from commercially available equipment (e.g., Donard and Weber 1985).

RECOMMENDED INSTRUMENTAL METHODS

The LODs recommended in Appendix A were arrived at by matching U.S. EPA water quality criteria (for water analyses) and the lowest concentrations expected in Puget Sound sediments and marine tissues against the detection limits possible for each matrix using agency-approved instrumental methods.

For mercury, the only instrumental method adequate for these requirements for all matrices is cold vapor atomic absorption (CVAA). For tissue, graphite furnace atomic absorption (GFAA) is required for all the other priority pollutant metals of concern.

For sediment, inductively-coupled plasma emission spectroscopy (ICP) methods are adequate for copper, nickel, and zinc. All other priority pollutant metals of concern in sediment require GFAA to achieve the LODs.

For water, ICP methods are adequate for cadmium and zinc. All other priority pollutant metals of concern in water require GFAA to achieve the LODs.

Atomic absorption analysis via hydride generation (HYDAA) can lower the detection limits for antimony, arsenic, and selenium by a factor of 10-100 (Skoog 1985). This technique may be substituted for GFAA for samples in which low concentrations of these three elements are expected, or it may be used routinely. The U.S. EPA-approved method for arsenic by hydride generation (U.S. EPA 1979a) is included in Appendix G and can be adapted for the analysis of selenium and antimony. Hydride generation may be coupled to ICP instead of AA for certain elements (e.g., selenium and antimony).

However, this method may be used only if accuracy and precision can be demonstrated to the levels specified in the Quality Assurance/Quality Control section of this report.

The U.S. EPA approved methods for GFAA and ICP analysis for all the metals of concern except mercury and for the CVAA analysis of mercury are detailed in Exhibit D of Appendix B (U.S. EPA 1985). It must be emphasized that GFAA requires duplicate injections and matrix spikes. If spike recovery is not within specific limits as detailed in the CLP (Exhibit E of Appendix B), the method of standard additions (MSA) may be required. The monitoring of GFAA spike recoveries is critical for all low level samples and is especially critical for the analysis of lead.

The use of matrix modifiers has become widespread in the GFAA analysis of freshwater (Manning and Slavin 1983), saltwater (Slavin et al. 1984, Manning and Slavin 1978), and biological samples (Hinderberger et al. 1981). No agency-approved protocol was found. However, matrix modifiers may be used, especially for lead, arsenic, selenium, and antimony, as long as accuracy and precision can be demonstrated to the levels specified in the Quality Assurance/Quality Control section of this report.

Although X-ray fluorescence (XFS) may achieve the LODs listed in Appendix A, no agency-approved method was found. XFS may be used if accuracy and precision can be demonstrated to the levels specified in the Quality Assurance/Quality Control section of this report.

Other agency-approved instrumental techniques with detection limits higher than those specified in Appendix A (e.g., direct flame atomic absorption) may be used only if the sample concentration exceeds twice the documented detection limit of the method. These U.S. EPA-approved methods are detailed in Appendix G (U.S. EPA 1979a).

METALS SPECIATION

Because the techniques for the analysis of arsenic and organotin species are not widely used, are not routine, and have inadequate QA/QC documentation, no protocols were recommended at the January PSDDA/PSEP workshop. Although not yet tested, it was suggested that methyl mercury may possibly be extracted by vacuum distillation (e.g., Hiatt and Jones 1984) and then analyzed by gas chromatography/mass spectrometry.

QUALITY ASSURANCE/QUALITY CONTROL

QA/QC MEASURES INITIATED BY THE ANALYTICAL LABORATORY

Standard laboratory practices for cleanliness as applied to glassware, reagents, solvents, gases, and instruments must be followed. For additional guidelines not covered in this report, see Sections 4 and 5 of Handbook for Analytical Quality Control in Water and Wastewater Laboratories (U.S. EPA 1979b).

Instrumental QA/QC Checks

Instrumental QA/QC checks necessary for all the U.S. EPA-approved methods discussed in the previous section include:

- Calibration blank
- Initial calibration and calibration verification
- Continuing calibration verification
- U.S. EPA Standard Reference Samples
- ICP interference check sample analysis (for ICP only).

Guidelines for instrumental calibration are given in U.S. EPA Methods for Chemical Analysis of Water and Wastes (U.S. EPA 1979a). In general, all instruments must be calibrated daily and each time the instrument is set up. Calibration procedures will follow the procedures specified for each analysis in the U.S. EPA protocols. In addition (as specified for the CLP, Appendix B), after an instrument system has been calibrated, the accuracy of the initial calibration shall be verified and documented for every analyte by the analysis of U.S. EPA Quality Control Solutions. Where a certified solution of an analyte is not available from U.S. EPA or any source, analyses shall be conducted on an independent standard at a concentration other than that used for calibration, but within the calibration range (e.g., tin is not currently present in U.S. EPA Quality Control Samples). When measurements for the certified components exceed the control limits, the analysis must be terminated, the problem corrected, the instrument recalibrated, and the calibration reverified.

For ICP and AA analyses, all work shall be performed using continuing calibration as outlined in the CLP Statement of Work with regard to Exhibit E, Sections 1 and 2 (see Appendix B). Frequency is 10 percent or every 2 h during an analysis run, whichever is more frequent.

Method QA/QC Checks

Contract laboratories should perform the quality control checks listed below:

- Preparation blank
- Spiked sample analysis
- Replicate sample analysis
- GFAA method of standard addition (if necessary)
- Laboratory control sample analysis (Standard Reference Materials).

Details on the use and application of these checks along with control limits, reporting requirements, and corrective actions are discussed in Exhibit E of Appendix B (U.S. EPA 1985). The frequencies of application of these checks are 5 percent or one per batch, whichever is more frequent. The control limits are ±20 percent relative percent difference for duplicates, 75-125 percent recovery for spikes, and 80-120 percent recovery for the analysis of standard reference materials (SRMs). For the purpose of QA/QC, the contract required detection limits (CRDLs) shall be those listed in Table 6 of Appendix A. For batches of five samples or less, the minimum QC checks should be a preparation blank and the analysis of an SRM. If an analyte is not in the SRM, a matrix spike must be analyzed for that particular analyte.

In general for small batches (i.e., ≤five samples), the priority of QC checks should be: SRMs > analytical duplicates > matrix spikes. If several small batches of the same matrix are analyzed sequentially (i.e., for several small projects), an SRM can be analyzed at a frequency of 5 percent overall, with at least one sample duplicate analyzed per individual batch. If any QA/QC check does not meet the established criteria, the laboratory QA officer should notify the project QA coordinator and the methods should be adjusted or, if necessary, data qualifications and reasons for noncompliance to criteria should be submitted with the analytical data report.

QA/QC MEASURES INITIATED IN THE FIELD

In addition to the QA/QC checks listed above, the following four checks may be initiated at the time of sample collection (Plumb 1981). These checks may not replace any of the QA/QC measures outlined above but may be included as part of the overall QA/QC program.

Transfer (Preservation) Blanks

Reagents can become contaminated after a period of use in the field. The transfer blank will shown this and any contamination introduced during shipping.

A sample container is filled with distilled water to the same volume as that for samples and preserved as if it were a normal water or sediment sample. This blank is then sent to the laboratory for analysis.

Cross-Contamination Blanks

Field equipment can exhibit carryover from one sample to the next if not thoroughly cleaned between sampling. The cross-contamination blank is designed to verify the absence of carryover.

Decontaminated sample-handling equipment (spatulas, augers, core barrels) is wiped with a clean lab tissue, which is then placed in a sealable container. Alternatively, the equipment is rinsed with distilled/deionized water, and the water is collected and preserved as if it were a normal water sample.

Blind Replicate Samples

A collected sample is homogenized and split in the field into at least three identical aliquots, and each aliquot is treated and identified as a separate sample. The replicates are sent blind to the laboratory. The mean, standard deviation, and relative percent standard deviation are calculated by the project QA coordinator.

In addition, a collected sample may be split in the field into two aliquots, and one aliquot sent for analysis to a different or "reference" laboratory. The relative percent difference is calculated by the project QA coordinator. If project constraints require the use of more than one laboratory, their comparability must be established using certified reference materials.

Blind Standard Reference Materials

A standard reference material is placed in a sample container at the time of sample collection and sent blind to the laboratory. The percent recovery is calculated by the project QA coordinator.

It is recommended that the same control limits be established as for the laboratory QA/QC checks. The project QA coordinator must inform the laboratory if the control limits are exceeded and corrective actions must be taken.

CORRECTIVE ACTIONS

If the concentration of the field or laboratory blank is greater than the required detection limit, all steps in the sample handling should be reviewed. Many trace metal contamination problems are due to airborne dust. Keeping containers closed, and rinsing all handling equipment immediately before use minimizes dust problems. In the field, mercury-filled thermometers are a potential source of severe mercury contamination. In the laboratory, samples for mercury analysis should be isolated from items such as polarographs or COD reagents.

Poor replication may be caused by inadequate mixing of the sample before taking aliquots, inconsistent contamination, inconsistent digestion procedures, or instrumentation problems. Instrumentation problems may be isolated by recalibration and calibration blank analysis. Inconsistent digestion may be due to analyte loss; see below for corrective action. Also, hotplates may not hold a constant temperature across their surfaces. This can be remedied by changing the position of digestion vessels at regular intervals during heating.

Poor performance on the analysis of the Standard Reference Material (SRM) or poor spike recovery may be caused for the same reasons as poor replication. However, if replicate results are acceptable, poor SRM performance or poor spike recovery may be caused by loss of analyte during digestion. To check for analyte loss during digestion and for low recovery due to interferences during analysis, spike the sample after digestion and compare the analysis to the predigestion spike. If the results are different, the digestion technique should be adjusted. If the results are the same, dilute the sample by at least a factor of five and reanalyze. If spike recovery is still poor, standard additions, matrix modifiers, or another method is required.

DATA REPORTING

Concentrations of elements from sediment samples should be reported on a dry weight basis. Those from tissue samples should be reported on a wet weight basis along with the percent moisture content (wet/dry ratio) of the tissue. If the tissue sample is too small to do both a metals analysis and a moisture determination, omit the latter.

DATA REPORT PACKAGE

The data report package for analyses of each sample should include:

- Tabulated results in units as specified for each matrix in the analytical protocols (Appendices B, D, E, and G), validated and signed in original by the laboratory manager
- Any data qualifications and explanation for any variance from the analytical protocols
- Results for all of the QA/QC checks initiated by the laboratory
- Tabulation of instrument and method detection limits.

BACKUP DOCUMENTATION

All contract laboratories are required to submit results that are supported by sufficient backup data and quality assurance results to enable independent QA reviewers to conclusively determine the quality of the data.

The laboratories should be able to supply legible photocopies of original data sheets with sufficient information to unequivocally identify:

- Calibration results
- Calibration and preparation blanks
- Samples and dilutions
- Duplicates and spikes
- Any anomalies in instrument performance or unusual instrumental adjustments.

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APPENDIX A

FINAL REPORT
TASK A-1

SELECTION OF METALS FOR PROTOCOL DEVELOPMENT

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TASK A-1
SELECTION OF METALS FOR PROTOCOL DEVELOPMENT

INTRODUCTION

Recent studies have documented high concentrations of a number of U.S. EPA priority pollutant metals and metalloids in the sediments of industrialized embayments in Puget Sound relative to reference areas. To ensure the acceptability of dredging and dredged material disposal, federal and state agencies must be able to accurately and routinely analyze for these substances in water, sediment, and biological samples. Because of the number of agencies involved and different program objectives, a standardized protocol is necessary to ensure comparability of results. It is the objective of Task A to develop this protocol.

A consensus approach involving federal, state, and local institutions will be used to develop both standardized chemical tests and standardized test interpretation guidelines for use by all Puget Sound regulatory agencies concerned with dredged materials. Protocols developed for each metal (or group of metals that can be analyzed simultaneously) will include guidance for field sampling, analytical procedures, and quality control requirements. When possible, existing procedures are to be recommended, and modified as appropriate. Draft protocols will be reviewed by a workshop of experts to reach a consensus on the recommended requirements. After incorporation of the workshop comments, a final protocol report will be drafted for final review.

The purpose of Task A-1 is to:

- Recommend appropriate metals and metalloids for which analytical protocols will be proposed under the Puget Sound Dredged Disposal Analysis (PSDDA) program

- Recommend limits of detection (LOD) for the analysis of each element in water, sediment, and tissue samples.

The thirteen U.S. EPA priority pollutant metals include three elements (antimony, arsenic, and selenium) that are classified as metalloids, which are elements that do not strictly occur as metals in the environment. Following U.S. EPA convention and for ease of discussion, all of these elements will be referred to as metals.

EVALUATION OF U.S. EPA PRIORITY POLLUTANT METALS

Protocols were requested by PSDDA for all metals either documented or likely to be of concern in Puget Sound. In addition, justification was required for the exclusion of any U.S. EPA priority pollutant metal.

Reports for four recent field investigations in Puget Sound were reviewed during the selection process (Malins et al. 1980, 1982; Galvin et al. 1984; Battelle 1985; Tetra Tech 1985a). Historical data from previous investigations summarized by Dexter et al. (1981) and recommendations for metals of concern proposed in several summary reports of contamination in Puget Sound were also reviewed (Konasewich et al. 1982; Jones and Stokes 1983; Quinlan et al. 1985).

The thirteen U.S. EPA priority pollutant metals and data reports providing evidence of their potential concern in Puget Sound are listed in Table 1. Criteria typically used to determine if a metal is of concern include high toxicity (measured in laboratory studies), high concentration relative to sediments from reference areas located away from industrialized areas of Puget Sound, and widespread distribution in Puget Sound. While all U.S. EPA priority pollutant metals are considered toxic, metals identified to be of concern have been found in high concentration only in limited areas of the Sound (e.g., antimony, arsenic, cadmium, copper, lead, mercury, and zinc at the Fourmile Rock disposal site relative to the rest of central Puget Sound; Galvin et al. 1984).

TABLE 1. METALS OF CONCERN AS INDICATED BY PUGET SOUND FIELD STUDIES

U.S. EPA Priority Pollutant Metal	Dexter et. al. 1981*	Malins et. al. 1982	Battelle 1985	Galvin et. al. 1984	Tetra Tech 1985a	Frequency of Concern (Out of 5)
Antimony (Sb)	x			x	x	3
Arsenic (As)	x	x	x	x	x	5
Beryllium (Be)						0
Cadmium (Cd)			x	x	x	3
Chromium (Cr)						0
Copper (Cu)	x	x	x	x	x	5
Lead (Pb)	x	x	x	x	x	5
Mercury (Hg)	x	x	x	x	x	5
Nickel (Ni)					x	1
Selenium (Se)		x				1
Silver (Ag)		x	x		x	3
Thallium (Tl)						0
Zinc (Zn)	x	x	x	x	x	5

* Secondary source (summarizes pre-1980 historical data).

Two areas of consensus are indicated by Table 1. First, three metals (beryllium, chromium, and thallium) were not identified by any report as of concern. Although potentially toxic, these metals are not present in Puget Sound sediments in concentrations that exceed natural levels. These three metals are not recommended for protocol development. Second, five metals (arsenic, copper, lead, mercury, and zinc) were identified in all reports as of concern because of their high concentration in urban embayments. A potential association with adverse biological effects was also noted in some reports. These five metals are recommended for protocol development.

Three additional metals (silver, cadmium, and antimony) were found in elevated concentration by a majority of the investigators. Of these metals, silver was found in high concentrations at relatively few sites (Malins et al. 1982; Battelle 1985; Tetra Tech 1985a). Silver is extremely toxic even at low concentrations, it is a potentially useful tracer of sewage effluent, and historically its concentration in Puget Sound sediments has increased faster than that of other metal pollutants (Crecelius, E., 22 October 1985, personal communication). Because of these factors, silver has been identified as a metal of concern. Cadmium is also highly toxic and is recommended for protocol development. Although antimony is less toxic, its high concentration in selected areas (e.g., sediments contaminated with copper smelter slag) warrants its inclusion.

Selenium was reported in high concentration in only one investigation (Malins et al. 1982). Several summary reports subsequently used these data to justify including selenium as a metal of concern (Konasewich et al. 1982; Jones and Stokes 1983; Quinlan et al. 1985). The instrumental technique used to measure selenium in the investigation (ion-coupled plasma emission spectroscopy) is subject to substantial spectral interferences (Parson et al. 1980). All other investigations using alternative techniques (e.g., graphite furnace atomic adsorption) have reported uniformly low or undetected concentrations of selenium. Therefore, selenium is not recommended for protocol development.

In Commencement Bay, nickel was found in somewhat elevated concentrations at a few sites exhibiting biological effects (Tetra Tech 1985a). Although

the available data suggest that nickel primarily derives from natural sources, there are insufficient data to dismiss nickel as a potential metal of concern in those few areas. Therefore, nickel is recommended for protocol development.

METALS SPECIATION

The toxicity of metals is highly dependent upon their chemical form (speciation). For example, inorganic arsenic in the +3 oxidation state is much more toxic than in the +5 oxidation state. Inorganic arsenic is toxic if ingested by humans, yet organic arsenic compounds are relatively nontoxic to mammals. In contrast, mercury salts are relatively nontoxic because of low solubilities, but methylated mercury compounds are highly toxic. Hence, quantitative determinations of different metal species could seriously affect interpretation of dredged material test results.

Recent studies (e.g., Crecelius et al. 1984; U.S. EPA 1980) have shown that arsenic and mercury exist predominantly as methylated compounds in marine biota. Dominance by one chemical form reduces the need for quantitative speciation studies as routine measurements. However, in some cases (e.g., health risk assessment) a precise determination of metals species in different bioaccumulation studies may be necessary.

In the water column, total concentrations of arsenic and mercury are low because of dilution, and in sediments, matrix effects confound speciation studies. Speciation techniques in general are difficult, time consuming, and nonroutine (Crecelius et al. 1984). Because of the apparent small incremental gain in knowledge from speciation analyses in biota for all but a few applications, routine analyses do not appear to be cost effective. Development of procedures for speciation analyses to be used in special circumstances will require a consensus from a workshop of experts, to be held as part of Task A.

EVALUATION OF NON-PRIORITY POLLUTANT METALS

None of the reports reviewed included a metal of concern that was not already on the U.S. EPA priority pollutant list. However, selected

non-priority pollutant metals could be of use in studies of dredged material. Their value in determining sediment quality values is being investigated as part of a separate task for PSDDA and the Puget Sound Estuarine Program.

Chemical data can be normalized to account for physical and physicochemical differences among samples. These differences may mask trends in chemical concentrations that are useful in interpreting environmental data. Metals from pollution sources tend to be selectively enriched within fine-grained sediment fractions having a high content of hydrous manganese or iron oxides. The incorporation of metals in these oxides may also affect their toxicity. Therefore, normalization of metal concentrations to the iron or manganese content of sediments may be useful for determining sediment quality values. It may also help account for the dilution of contaminated sediments by materials from unrelated sources during transport and deposition.

Manganese and iron are recommended for inclusion in the metals protocol.

RECOMMENDED LIMITS OF DETECTION

Environmental analytical chemists have not universally agreed upon a convention for determining and reporting the lower detection limits of analytical procedures. Furthermore, the basis for detection limits reported in the literature is rarely given. Values reported as lower detection limits are commonly based on instrumental sensitivity, levels of blank contamination, and/or matrix interferences and have various levels of statistical significance. The American Chemical Society's Committee on Environmental Improvement (CEI) defined the following types of detection limits in an effort to standardize the reporting procedures of environmental laboratories (Keith et al. 1983):

- Instrument Detection Limit (IDL) -- the smallest signal above background noise that an instrument can detect reliably.

- Limit of Detection (LOD) -- the lowest concentration level that can be determined to be statistically different from

the blank. The recommended value for LOD is 3σ, where σ is the standard deviation of the blank in replicate analyses.

- Limit of Quantitation (LOQ) — the level above which quantitative results may be obtained with a specified degree of confidence. The recommended value for LOQ is 10σ, where σ is the standard deviation of blanks in replicate analyses.

- Method Detection Limit (MDL) -- the minimum concentration of a substance that can be identified, measured, and reported with 99 percent confidence that the analyte concentration is greater than zero. The MDL is determined from seven replicate analyses of a sample of a given matrix containing the analyte (Glaser et al. 1981).

The CEI recommended that results below 3σ should be reported as "not detected" (ND) and that the detection limit (or LOD) be given in parentheses. In addition, if the results are near the detection limit (3σ to 10σ, which is the "region of less-certain quantitation"), the results should be reported as detections with the limit of detection given in parentheses.

The CEI definitions are useful for establishing a conceptual framework for detection limits, but are somewhat limited in a practical sense. The IDL does not address possible blank contaminants or matrix interferences and is not a good standard for complex environmental matrices, such as tissues. The LOD and LOQ account for blank contamination, but not for matrix complexity and interferences. The high 10σ level specified for LOQ helps to preclude false positive findings, but may also necessitate the rejection of valid data. The MDL is the only operationally defined detection limit and provides a high statistical confidence level but, like the LOQ, may be too stringent and necessitate the rejection of valid data.

The detection limits recommended in this report are not strictly based on the CEI definitions. Instead, they are considered to be typically attainable values based on the best professional judgment and experience of analytical chemists who considered the instrumental sensitivity of affordable equipment,

common problems with blank contamination and matrix interferences, and reasonable levels of laboratory analytical effort. The recommended values are not absolute, as analytical procedures and laboratory precision can affect attainable detection levels. The detection limits recommended herein fall between the IDL and MDL as defined by the CEI.

The LOD for metals selected for protocol development must be specified low enough so that data from the analysis of a particular matrix (i.e., water, sediment, or biota) will be comparable and of acceptable quality. LOD reported for the recommended metals are summarized in Table 2 for each matrix type analyzed by a variety of instrumental techniques: direct flame AA (DFAA), graphite furnace AA (GFAA), cold vapor AA (CVAA), and ion-coupled plasma emission spectroscopy (ICP).

Several factors affect achievable detection limits regardless of the analytical procedure. These include the available sample size, presence of interfering substances, range of pollutants to be analyzed, and level of pollutant introduced as a contaminant during sampling or analysis. LOD given in Table 2 are provided as examples of typical attainable levels. These limits are not intrinsically constant for a particular method, but also depend on the precision attainable by an individual laboratory. Each laboratory must evaluate its own precision and thus establish its own detection limits. Large variations from the LOD in Table 2 may occur depending on the analytical instrumentation and technique and on individual sample characteristics.

Recommendations of LOD must be based, in part, on the analytical requirements to quantify concentrations found in relatively "clean" reference areas, and to approach water criteria levels. Table 3 lists the U.S. EPA water quality criteria. The range of concentrations of the recommended metals in sediments from Puget Sound reference areas is shown in Table 4. The range of detection limits reported for these metals in marine tissue samples is shown in Table 5. Recommended LOD are summarized in Table 6. The recommended LOD for sediments and tissues were selected by considering attainable LOD for different instruments (Table 2), U.S. EPA water quality criteria (Table 3), and reported environmental levels (Tables 4 and 5).

TABLE 2. LIMITS OF DETECTION FOR WATER, SEDIMENT, AND TISSUE MATRICES BY INSTRUMENT

		Water[a]			Sediment[b]		Tissue[c]		
		ICP	DFAA	GFAA	ICP	GFAA	ICP	DFAA	GFAA
Antimony	(Sb)	0.032	0.2	0.003	3.2	0.1	10	--	0.02
Arsenic	(As)	0.053	--	0.001	--	0.1	3	--	0.02
Cadmium	(Cd)	0.004	0.005	0.0001	4.0	0.1	0.4	0.1	0.01
Copper	(Cu)	0.006	0.02	0.001	0.6	0.1	0.6	0.1	0.01
Iron	(Fe)	NA[d]	NA	NA	0.7	--	NA	NA	NA
Lead	(Pb)	0.042	0.1	0.001	4.2	0.1	4	1.0	0.03
Mercury	(Hg)	0.0002 (CVAA)			0.01 (CVAA)		0.01 (CVAA)		
Manganese	(Mn)	NA	NA	NA	2.0	--	NA	NA	NA
Nickel	(Ni)	0.015	0.04	0.001	1.5	0.1	1	0.5	0.02
Silver	(Ag)	0.002	0.01	0.0002	0.7	0.1	0.7	0.1	0.01
Zinc	(Zn)	0.007	0.005	0.00005	0.2	0.2	0.2	0.1	0.2

[a] DFAA and GFAA data from U.S. EPA 1979; ICP data from U.S. EPA 1984. Values are mg/L.

[b] ICP data from Tetra Tech 1984; GFAA and CVAA data are detection limits that can be reasonably attained by various laboratories. Under strict conditions these limits can be lowered (e.g., Battelle 1985). Values are mg/kg dry weight for 5 g (wet) sediment in a 100 mL digest.

[c] Tetra Tech 1985b. Values are ug/g wet weight for 5 g tissue in a 50 mL digest.

[d] NA = Not applicable; iron and manganese used as natural tracers for sediments only.

TABLE 3. U.S. EPA WATER QUALITY CRITERIA[a] (ug/L)

Metal	EPA WQC
Antimony	--
Arsenic	36
Cadmium	9.3
Copper	2.9
Lead	5.6
Mercury	0.025
Nickel	7.1
Silver	2.3
Zinc	58

[a] U.S. EPA 1980a and U.S. EPA 1985.

TABLE 4. SUMMARY OF METAL CONCENTRATIONS IN SEDIMENTS FROM PUGET SOUND REFERENCE AREAS

	Range (mg/kg dry wt)	Mean (mg/kg dry wt)	Detection Frequency	Reference Sites[a]
Antimony	U 0.1[b] - 1.7	0.32[c] - 0.38[d]	12/32	1,2,3,4,7,8,9
Arsenic	1.9 - 17	7.2	34/34	1,2,3,4,7,8,9
Cadmium	0.1 - 1.9	0.67	24/24	1,2,3,4,6,9
Copper	5 - 74	32	28/28	1,2,3,4,5,6,9
Lead	U 0.1 - 24	9.8[c] - 9.8[d]	21/28	1,2,3,4,5,6,9
Mercury	0.01 - 0.28	0.08	38/38	1-9
Nickel	4 - 47	28	26/26	1,2,3,4,5,9
Silver	0.02 - 3.3	1.2	26/26	1,2,3,4,5,9
Zinc	15 - 100	62	26/26	1,2,3,4,5,9

[a] Reference sites: 1. Carr Inlet 2. Samish Bay 3. Dabob Bay 4. Case Inlet 5. Port Madison 6. Port Susan 7. Nisqually Delta 8. Hood Canal 9. Sequim Bay

[b] U: Undetected at the limit of detection shown.

[c] Mean calculated using 0.00 for undetected values.

[d] Mean calculated using the reported detection limit for undetected values.

References:

(Site 1) Tetra Tech 1985a; Crecelius et al. (1975).
(Site 2) Battelle (1985).
(Site 3) Battelle (1985).
(Site 4) Crecelius et al. (1975); Malins et al. (1980).
(Site 5) Malins et al. (1980).
(Site 6) Malins et al. (1981).
(Site 7) Crecelius et al. (1975).
(Site 8) Crecelius et al. (1975).
(Site 9) Battelle (1985).

TABLE 5. MINIMUM AND MAXIMUM METAL DETECTION LIMITS REPORTED FOR TISSUE SAMPLES (FROM TETRA TECH 1985b)

Element	Detection Limit (ug/g-wet weight)	
	Minimum	Maximum
Antimony	0.01	1.0
Arsenic	Always detected (minimum = 0.72)	
Cadmium	0.001	0.75
Copper	Always detected (minimum = 0.052)	
Lead	0.030	1.6
Mercury	0.0004	0.09
Nickel	0.019	1.0
Silver	0.001	0.27
Zinc	Always detected (minimum = 1.42)	

TABLE 6. RECOMMENDED LIMITS OF DETECTION FOR WATER, SEDIMENT, AND TISSUE MATRICES

	Water[a]	Sediment[b]	Tissue[c]
Antimony	3	0.1	0.02
Arsenic	1	0.1	0.02
Cadmium	0.1	0.1	0.01
Copper	1	0.1	0.01
Iron	NA[d]	0.7	NA
Lead	1	0.1	0.03
Mercury	0.2[e]	0.01	0.01
Manganese	NA	2.0	NA
Nickel	1	0.1	0.02
Silver	0.2	0.1	0.01
Zinc	1	0.2	0.2

[a] ug/L.

[b] mg/kg dry weight.

[c] ug/g wet weight.

[d] NA = Not applicable; iron and manganese used as natural tracers for sediments only.

[e] This detection limit can be lowered to 0.02 ug/L by using a special, gold-amalgamation-cold-vapor technique (Bloom and Crecelius 1983). However, accuracy and precision must be demonstrated to the levels specified in the QA/QC section of the main body of this report.

Recommended LOD for water are for graphite furnace analysis, except for cold vapor atomic adsorption for mercury.

SUMMARY

U.S. EPA priority pollutant metals recommended for inclusion in protocol development include antimony, arsenic, cadmium, copper, lead, mercury, nickel, silver, and zinc. U.S. EPA priority pollutant metals excluded include beryllium, chromium, selenium, and thallium. Speciation protocols may be useful for selected bioaccumulation studies (i.e., arsenic and mercury), but are not recommended for routine monitoring, or for analysis of waters or sediments. Iron and manganese are recommended for inclusion in the protocol specification because of their potential use as geochemical tracers and as potentially important factors in the generation of sediment quality values.

The list of metals recommended for the development of sediment quality values (SQV) in Puget Sound (Tetra Tech 1985c) includes all 13 priority pollutant metals. These were presented only as potential candidates for metals of concern. The purpose of the SQV report in this regard was to provide guidance for selecting contaminants of concern rather than to actually select the contaminants. Thus, the SQV list contains some metals specifically excluded in this report.

Limits of detection are recommended for the U.S. EPA priority pollutant metals after a consideration of attainable detection limits for each matrix type and expected levels in the environment (sediments and tissues). Based on this comparison, maximum detection limits (often meaning lower cost) are recommended that would still be sufficient for characterizing each matrix in a dredged materials testing program. Detection limits for speciation analyses (e.g., inorganic arsenic or methylmercury in tissues) are roughly similar to those attainable for the total metal analysis.

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APPENDIX B

EXCERPTS FROM

EXHIBIT B: REPORTING REQUIREMENTS AND DELIVERABLES

EXHIBIT D: ANALYTICAL METHODS

EXHIBIT E: QUALITY ASSURANCE/QUALITY CONTROL

EXHIBIT G: CHAIN-OF-CUSTODY AND DOCUMENT CONTROL PROCEDURES

(U.S. EPA 1985)

CONTRACT LABORATORY PROGRAM

STATEMENT OF WORK
(SOW)

INORGANIC ANALYSIS
Multi-Media
Multi-Concentration

SOW No. 785
July 1985

Based on: Caucus Inorganics Protocol

EXHIBIT B

REPORTING REQUIREMENTS AND DELIVERABLES

REPORTING REQUIREMENTS AND DELIVERABLES

The contractor shall provide reports and other deliverables specified hereunder. Specific reports are described below. The Contract Reporting Schedule which follows the report descriptions gives delivery schedule and report distribution information, including addresses for report designees. All reports to multiple addresses must be sent out concurrently by whatever method necessary to meet the contract required time limits. All reports must also be submitted in a legible form or resubmission will be required at no additional cost to the Agency.

Report Description

A. Weekly Progress Reports - Tabulation of samples received, date of receipt, and a tabulation of problems encountered.

B. Sample Traffic Report (Original Lab Copy for Return to SMO) - Copy of SMO Sample Traffic Report with lab receipt information and original Contractor signature.

C. Sample Data Package - Data report package for analyses of each sample (including all required QA/QC-Exhibit E) must be complete before submission and shall include:

1) Copies of completed SMO Sample Traffic Reports with receipt of information completed for all samples reported in data package.

2) The cover page for the inorganic analysis data package (Exhibit B) including general comments, Statement of Work (SOW) Number, QC Report #, sample EPA cross reference numbers, footnotes used in the data package, and the statement on use of ICP background and interelement corrections for the samples. The SOW number defines the Statement of Work used to obtain the reported values.

3) Tabulated results in ug/L for aqueous samples or mg/kg for solid samples (identification and quantity) of specified chemical constituents (Exhibit C) by the specified analyses (Exhibit D), validated and signed in original signature by the Laboratory Manager, and reported on Form I.* The results for solid samples will be reported on a dry weight basis. Percent solids are not required on aqueous samples. If the value or the result is greater than or equal to the Instrument Detection Limit (IDL), corrected for dilutions, as determined in Exhibit E, report the value and indicate the analytical method used for the metals. Use P for ICP, A for flame AA and F for furnace AA. All dilutions not required by the contract and affecting the IDL, must be noted on an element by element basis on Form I. If the value is less than the Contract Required Detection Limits (CRDL) in Exhibit C, put the value in brackets, e.g. [10]. If the element was analyzed for but not detected, report the instrument detection limit value with a "U"

*In the event the Laboratory Manager cannot validate all data reported for each sample, he/she will provide a detailed description of the problems associated with the sample.

(e.g., 10U). Use an "E" as the footnote to indicate an estimated value or value not reported due to the presence of interference, and an explanatory note must be included on the cover page. If the duplicate sample analysis is not within the control limits, flag it with an asterisk (*). Use "S" as a footnote to indicate a value determined by Method of Standard Additions (MSA). If the correlation coefficient (r) for method of standard additions is less than 0.995, flag the value with a '+' sign. If duplicate injection precision criteria specified for Furnace AA analysis in Exhibit E cannot be met, flag the data with an "M". If the spike sample recovery is not within control limits, flag the data with the letter N. Report results to two significant figures for values from 0 to 100 and three significant figures for results greater than 100, with the exception of Mercury (see Mercury Methods - Exhibit D). For rounding rules, follow the EPA Handbook of Analytical Quality Control in Water and Wastewater Laboratories (EPA-600/4-79-019).

Under the comments section on Form I, provide a brief physical description of the sample using the following guidelines:

A. Water samples - Coloration and clarity

B. Solid samples - Coloration, texture and artifacts

Recommended Descriptive Terms

Coloration: Red, blue, yellow, green, orange, violet, white, colorless, brown, grey, black

Clarity: Clear, cloudy, opaque.

Texture: Fine (powdery), Medium (sand), Coarse (large crystals or rocks)

Also note any significant changes that occur during sample preparation (i.e. coloration shifts, emulsion formation).

4) Analytical results for samples and spikes, duplicates, standards, ICP Interference Check Samples, reagent blanks, laboratory control samples, instrument detection limits and holding times on QA Forms II, III, IV, V, VI, VII, VIII, IX and X. Multiple forms require identification (i.e. Form IIA, Form IIB etc.) Summarize each full method of standard addition performed on Form VIII.

5) Legible photocopy of raw data (sequential measurement readout record) clearly labeled with sufficient information to unequivocally identify:

a) calibration standards (including prep date).

b) calibration blanks and preparation blanks.

c) initial and continuing calibration verification standards and interference check samples.

d) diluted and undiluted samples (by EPA number) and all weights, dilutions and volumes used to obtain the reported values. If the volumes, weights and dilutions are consistent for all samples in a given Case, a general statement outlining these parameters is sufficient.

e) duplicates.

f) spikes (indicating standard solutions used, final spike concentrations, volumes involved).

g) any instrument adjustments, data corrections or other apparent anomalies on the measurement record including all data voided or data not used to obtain reported values.

h) all information for furnace analysis contained in Form VIII (Exhibit E), clearly identified on the raw data including sample #, initial single spike data, % recovery, full MSA data, MSA correlation coefficient, slope and intercept of linear fit, and final sample concentration (standard addition concentration).

6) Copies of digestion logs for the ICP, flameless AA and Hg preparations and of the distillation log for cyanide. These logs must include: 1) date, 2) sample weights and volumes, 3) sufficient information to unequivocably identify which QC samples (i.e. LCS, preparation blank) correspond to each batch digested, 4) comments describing any significant sample changes or reactions which occur during preparation, 5) indication of pH <2 or >12, as applicable.

7) The order of raw data in the data package shall be: 1) ICP, 2) Flame AA, 3) Furnace AA, 4) Hg, 5) CN, 6) Digestion and Distillation logs, 7) Percent Solids. All raw data must include intensities (ICP) and absorbances (AA) unless instrument direct readout is in concentration units.

8) For each reported value, the data package must contain all raw data from the instrument used to obtain that value and the QA/QC values reported. All instruments must provide a hard copy of the instrument readout (i.e. stripcharts, printer tapes, etc.). A photocopy of the instrument readout must be included in the raw data package.

Information shall include a key to abbreviations, with response units stated, and a cross reference to EPA sample numbers.

D. <u>Results of Intercomparison/Performance Evaluation(PE) Sample Analyses</u>

Tabulation of analytical results for Intercomparison/PE Sample analyses include all requirements specified in C above.

E. Complete Case File Purge (formerly termed the "Document Control and Chain-of-Custody Package")

The Complete Case File Purge package must include all laboratory records received or generated for a specific case that have not been previously submitted to EPA as deliverable. These items include but are not limited to: sample tags, custody records, sample tracking records, analysts logbook pages, bench sheets, instrument readout records, computer printouts, raw data summaries, instrument logbook pages (including instrument conditions), correspondence, and the document inventory.

Shipment of each Complete Case File Purge package by first class mail, overnight carrier, priority mail or equivalent is acceptable. Custody seals, which are provided by EPA, must be placed on shipping containers and a document inventory and transmittal letter included. The laboratory is not required to maintain any documents for a case after shipment; however, it is recommended that you maintain a copy of the document inventory and transmittal letter.

F. Quarterly Verification of Instrument Parameters

The Contractor must perform and report quarterly verification of instrument detection limits by methods specified in Exhibit E and report type and model # for each instrument used on this contract. For the ICP instrumentation and methods, the Contractor must also report quarterly: linearity range verification, interelement correction factors, wavelengths used and integration times. This information is reported using Forms XI, XII and XIII. Submissions of Quarterly Verification of Instrument Parameters must include the raw data used to determine those values reported.

G. Results of Solid Laboratory Control Samples (LCS)

Tabulation of analytical results and QC for the solid LCS sample analysis, as specified in Exhibit E.

CONTRACT REPORTING SCHEDULE

Report	No. Copies	Delivery Schedule*	SMO(1)	EMSL/ LV(2)	Region/ Client(3)	CEAT NEIC(4)
A. Weekly Progress Report	1	Weekly	X			
B. Sample Traffic Report	1	7 days from receipt of sample	X			
C. Sample Data Package	3	35 days from receipt of sample	X	X	X	
D. Results of Inter-comparison Study/ PE Sample Analysis	1	35 days from receipt of sample		X		
E. Compilation of Complete Case File Purge Package	1	7 days after data submission	- - - - - -	- - - - - -	N/A - - - - - -	- - - - - -
F. Complete Case File Purge Package	1	180 days after data submission or 7 days from request by PO or SMO				X
G. Quarterly Verification of Instrument Parameters	2	Quarterly: 15th day of January, April, July, October	X	X		
H. Results of Solid LCS Analysis	2	Monthly: 15th of every month	X	X		

*Schedule designated in calendar days.

REPORT DISTRIBUTION ADDRESSEES:

(1) USEPA
Contract Laboratory Program
Sample Management Office (SMO)
P.O. Box 818
Alexandria, VA 22313

For overnight delivery service, use street address:

300 N. Lee Street
Alexandria, VA 22314

(2) USEPA
Environmental Monitoring
Systems Laboratory (EMSL/LV)
P.O. Box 15027
Las Vegas, NV 89114
ATTN: Data Audit Staff

For overnight delivery service, use street address:

944 E. Harmon, Exec. Ctr. Rm. 226
Las Vegas, NV 89109
ATTN: Data Audit Staff

(3) USEPA REGIONS:

The CLP Sample Management Office will provide the contractor with the list of addresses for distribution to the ten EPA Regions. SMO will provide the Contractor with updated Regional address/name lists as necessary throughout the period of the contract.

(4) USEPA
NEIC Contract Evidence Audit Team
12600 West Colfax Ave., Suite C310
Lakewood, CO 80215

U.S. EPA Contract Laboratory Program
Sample Management Office
P.O. Box 818 - Alexandria, VA 22313
703/557-2490 FTS: 8-557-2490

Date _____

COVER PAGE
INORGANIC ANALYSES DATA PACKAGE

Lab Name _____ Case No. _____

SOW No. _____ Q.C. Report No. _____

Lab Receipt Date _____

Sample Numbers

EPA No.	Lab ID No.	EPA No.	Lab ID No.

Comments: _____

ICP interelement and background corrections applied? Yes ____ No ____.

If yes, corrections applied before _____ or after _____ generation of raw data.

Footnotes:

NR - Not required by contract at this time

Form I:

Value - If the result is a value greater than or equal to the instrument detecion limit but less than the contract-required detection limit, report the value in brackets (i.e., [10]). Indicate the analytical method used with P (for ICP), A (for Flame AA) or F (for Furnace AA).

U - Indicates element was analyzed for but not detected. Report with the instrument detection limit value (e.g., 10U).

E - Indicates a value estimated or not reported due to the presence of interference. Explanatory note included on cover page.

S - Indicates value determined by Method of Standard Addition.

N - Indicates spike sample recovery is not within control limits.

* - Indicates duplicate analysis is not within control limits.

\+ - Indicates the correlation coefficient for method of standard addition is less than 0.995

M - Indicates duplicate injection results exceeded control limits.

Indicate method used: P for ICP; A for Flame AA and F for Furnace.

Form I

U.S. EPA Contract Laboratory Program
Sample Management Office
P.O. Box 818 - Alexandria, VA 22313
703/557-2490 FTS: 8-557-2490

EPA Sample No. _____

Date _____

INORGANIC ANALYSIS DATA SHEET

LAB NAME _____ CASE NO. _____

SOW NO. _____

LAB SAMPLE ID. NO. _____ QC REPORT NO. _____

Elements Identified and Measured

Concentration: Low _____ Medium _____

Matrix: Water _____ Soil _____ Sludge _____ Other _____

ug/L or mg/kg dry weight (Circle One)

1.	Aluminum _____	13.	Magnesium _____
2.	Antimony _____	14.	Manganese _____
3.	Arsenic _____	15.	Mercury _____
4.	Barium _____	16.	Nickel _____
5.	Beryllium _____	17.	Potassium _____
6.	Cadmium _____	18.	Selenium _____
7.	Calcium _____	19.	Silver _____
8.	Chromium _____	20.	Sodium _____
9.	Cobalt _____	21.	Thallium _____
10.	Copper _____	22.	Vanadium _____
11.	Iron _____	23.	Zinc _____
12.	Lead _____		Precent Solids (%) _____
	Cyanide _____		

Footnotes: For reporting results to EPA, standard result qualifiers are used as defined on Cover Page. Additional flags or footnotes explaining results are encouraged. Definition of such flags must be explicit and contained on Cover Page, however.

Comments: _____

Lab Manager _____

Form II

Q. C. Report No. _____

INITIAL AND CONTINUING CALIBRATION VERIFICATION[3]

LAB NAME _____ CASE NO. _____

SOW NO. _____

DATE _____ UNITS _____

Compound	Initial Calib.[1]			Continuing Calibration[2]					
Metals:	True Value	Found	%R	True Value	Found	%R	Found	%R	Method[4]
1. Aluminum									
2. Antimony									
3. Arsenic									
4. Barium									
5. Beryllium									
6. Cadmium									
7. Calcium									
8. Chromium									
Cobalt									
10. Copper									
11. Iron									
12. Lead									
13. Magnesium									
14. Manganese									
15. Mercury									
16. Nickel									
17. Potassium									
18. Selenium									
19. Silver									
20. Sodium									
21. Thallium									
22. Vanadium									
23. Zinc									
Other: _____									
Cyanide									

1 Initial Calibration Source _____ 2 Continuing Calibration Source _____

3 Control Limits: Mercury and Tin 80-120; Other Metals 90-110; Cyanide 85-115

4 Indicate Analytical Method Used: P - ICP; A - Flame AA; F - Furnace AA

Form III

Q. C. Report No. _____

BLANKS

LAB NAME _____ CASE NO. _____

DATE _____ UNITS _____

Matrix _____

Compound	Initial Calibration Blank Value	Continuing Calibration Blank Value 1	2	3	4	Preparation Blank Matrix: _____ 1	Matrix: _____ 2
Metals:							
1. Aluminum							
2. Antimony							
3. Arsenic							
4. Barium							
5. Beryllium							
6. Cadmium							
7. Calcium							
8. Chromium							
9. Cobalt							
10. Copper							
11. Iron							
12. Lead							
13. Magnesium							
14. Manganese							
15. Mercury							
16. Nickel							
17. Potassium							
18. Selenium							
19. Silver							
20. Sodium							
21. Thallium							
22. Vanadium							
23. Zinc							
Other:							
Cyanide							

Form IV

Q. C. Report No. _____

ICP INTERFERENCE CHECK SAMPLE

LAB NAME _____ CASE NO. _____

Check Sample I.D. _____

DATE _____ Check Sample Source _____

Units _____

Compound	Control Limits[1] Mean	Control Limits[1] Std. Dev.	True[2]	Initial Observed	Initial %R	Final Observed	Final %R
Metals:							
1. Aluminum							
2. Antimony							
3. Arsenic							
4. Barium							
5. Beryllium							
6. Cadmium							
7. Calcium							
8. Chromium							
. Cobalt							
10. Copper							
11. Iron							
12. Lead							
13. Magnesium							
14. Manganese							
15. Mercury							
16. Nickel							
17. Potassium							
18. Selenium							
19. Silver							
20. Sodium							
21. Thallium							
22. Vanadium							
23. Zinc							
Other: _____							

1 Mean value based on n = _____.

2 True value of EPA ICP Interference Check Sample or contractor standard.

Form V

Q. C. Report No. _____

SPIKE SAMPLE RECOVERY

LAB NAME _____

DATE _____

CASE NO. _____

EPA Sample No. _____

Lab Sample ID No. _____

Units _____

Matrix _____

Compound	Control Limit %R	Spiked Sample Result (SSR)	Sample Result (SR)	Spiked Added (SA)	%R[1]
Metals:					
1. Aluminum	75-125				
2. Antimony	"				
3. Arsenic	"				
4. Barium	"				
5. Beryllium	"				
6. Cadmium	"				
7. Calcium	"				
8. Chromium	"				
9. Cobalt	"				
10. Copper	"				
11. Iron	"				
12. Lead	"				
13. Magnesium	"				
14. Manganese	"				
15. Mercury	"				
16. Nickel	"				
17. Potassium	"				
18. Selenium	"				
19. Silver	"				
20. Sodium	"				
21. Thallium	"				
22. Vanadium	"				
23. Zinc	"				
Other:					
Cyanide	"				

[1] %R = [(SSR - SR)/SA] x 100

"N" - out of control

"NR" - Not required

Comments: _____

Form VI

Q. C. Report No. _____

DUPLICATES

LAB NAME _____

DATE _____

CASE NO. _____

EPA Sample No. _____

Lab Sample ID No. _____

Units _____

Matrix _____

Compound	Control Limit[1]	Sample(S)	Duplicate(D)	RPD[2]
Metals:				
1. Aluminum				
2. Antimony				
3. Arsenic				
4. Barium				
5. Beryllium				
6. Cadmium				
7. Calcium				
8. Chromium				
9. Cobalt				
). Copper				
11. Iron				
12. Lead				
13. Magnesium				
14. Manganese				
15. Mercury				
16. Nickel				
17. Potassium				
18. Selenium				
19. Silver				
20. Sodium				
21. Thallium				
22. Vanadium				
23. Zinc				
Other:				
yanide				

* Out of Control

[1] To be added at a later date.

[2] RPD = [|S - D|/((S + D)/2)] x 100

NC - Non calculable RPD due to value(s) less than CRDL

Form VII

Q.C. Report No. _____

INSTRUMENT DETECTION LIMITS AND

LABORATORY CONTROL SAMPLE

LAB NAME _____ CASE NO. _____ DATE _____

LCS NO. _____

Compound	Required Detection Limits (CRDL)-ug/l	Instrument Detection Limits (IDL)-ug/l ICP/AA ID# _____	Instrument Detection Limits (IDL)-ug/l Furnace ID# _____	Lab Control Sample ug/L mg/kg (circle one) True	Found	%R
Metals:						
1. Aluminum	200					
2. Antimony	60					
3. Arsenic	10					
4. Barium	200					
5. Beryllium	5					
6. Cadmium	5					
7. Calcium	5000					
8. Chromium	10					
9. Cobalt	50					
10. Copper	25					
11. Iron	100					
12. Lead	5					
13. Magnesium	5000					
14. Manganese	15					
15. Mercury	0.2					
16. Nickel	40					
17. Potassium	5000					
18. Selenium	5					
19. Silver	10					
20. Sodium	5000					
21. Thallium	10					
22. Vanadium	50					
23. Zinc	20					
Other:						
Cyanide	10	NR	NR			

NR - Not required

Form VIII

Q.C. Report No. _____

STANDARD ADDITION RESULTS

LAB NAME _____ CASE NO. _____

DATE _____ UNITS _____

EPA Sample #	Element	0 ADD ABS.	1 ADD		2 ADD		3 ADD		FINAL CON.[2]	r*
			CON.	ABS[1]	CON.	ABS.[1]	CON.	ABS.[1]		

1 CON is the concentration added, ABS. is the instrument readout in absorbance or concentration.

2 Concentration as determined by MSA

*"r" is the correlation coefficient.

+ - correlation coefficient is outside of control window of 0.995.

Form IX

Q. C. Report No. _____

ICP SERIAL DILUTIONS

LAB NAME _____ CASE NO. _____

EPA Sample No. _____

DATE _____ Lab Sample ID No. _____

Units _____

Matrix _____

Compound	Initial Sample Concentration(I)	Serial Dilution[1] Result(S)	% Difference[2]
Metals:			
1. Aluminum			
2. Antimony			
3. Arsenic			
4. Barium			
5. Beryllium			
6. Cadmium			
7. Calcium			
8. Chromium			
9. Cobalt			
10. Copper			
11. Iron			
12. Lead			
13. Magnesium			
14. Manganese			
15. Nickel			
16. Potassium			
17. Selenium			
18. Silver			
19. Sodium			
20. Thallium			
21. Vanadium			
22. Zinc			
Other:			

1 Diluted sample concentration corrected for 1:4 dilution (see Exhibit D)

2 Percent Difference = $\frac{|I - S|}{I} \times 100$

NR - Not Required, initial sample concentration less than 10 times IDL

NA - Not Applicable, analyte not determined by ICP

Form X

QC Report No. _____

HOLDING TIMES

LAB NAME _____

DATE _____ CASE NO. _____

EPA Sample No.	Matrix	Date Received	Mercury Prep Date	Mercury Holding Time (Days)	CN Prep Date	CN Holding Time (Days)

Form XI (Quarterly)

INSTRUMENT DETECTION LIMITS

LAB NAME _____ DATE _____

ICP/Flame AA (Circle One) Model Number_____ Furnace AA Number_____

Element	Wavelength (nm)	CRDL (ug/L)	IDL (ug/L)	Element	Wavelength (nm)	CRDL (ug/L)	IDL (ug/L)
1. Aluminum		200		13. Magnesium		5000	
2. Antimony		60		14. Manganese		15	
3. Arsenic		10		15. Mercury		0.2	
4. Barium		200		16. Nickel		40	
5. Beryllium		5		17. Potassium		5000	
6. Cadmium		5		18. Selenium		5	
7. Calcium		5000		19. Silver		10	
8. Chromium		10		20. Sodium		5000	
9. Cobalt		50		21. Thallium		10	
10. Copper		25		22. Vanadium		50	
11. Iron		100		23. Zinc		20	
12. Lead		5					

Footnotes:
- Indicate the instrument for which the IDL applies with a "P" (for ICP), an "A" (for Flame AA), or an "F" (for Furnace AA) behind the IDL value.
- Indicate elements commonly run with background correction (AA) with a "B" behind the analytical wavelength.
- If more than one ICP/Flame or Furnace AA is used, submit separate Forms XI-XIII for each instrument.

COMMENTS: _____

_____

_____

_____

Lab Manager _____

Form XII (Quarterly)

ICP Interelement Correction Factors

LABORATORY _____ ICP Model Number _____

DATE _____

		Interelement Correction Factors for							
Analyte	Analyte Wavelength (nm)	Al	Ca	Fe	Mg				
1. Antimony									
2. Arsenic									
3. Barium									
4. Beryllium									
5. Cadmium									
6. Chromium									
7. Cobalt									
8. Copper									
9. Lead									
10. Manganese									
11. Mercury									
12. Nickel									
13. Potassium									
14. Selenium									
15. Silver									
16. Sodium									
17. Thallium									
18. Vanadium									
19. Zinc									

COMMENTS: _____

_____

 Lab Manager _____

Form XII (Quarterly) (cont'd)

ICP Interelement Correction Factors

LABORATORY _____ ICP Model Number _____

DATE _____

		Interelement Correction Factors for							
Analyte	Analyte Wavelength (nm)								
1. Antimony									
2. Arsenic									
3. Barium									
4. Beryllium									
5. Cadmium									
6. Chromium									
7. Cobalt									
8. Copper									
9. Lead									
10. Manganese									
11. Mercury									
12. Nickel									
13. Potassium									
14. Selenium									
15. Silver									
16. Sodium									
17. Thallium									
18. Vanadium									
19. Zinc									

COMMENTS: _____

Lab Manager _____

Form XIII (Quarterly)
ICP Linear Ranges

LAB NAME _____ ICP Model Number _____

DATE _____

Analyte	Integration Time (Seconds)	Concen-tration (ug/L)	Analyte	Integration Time (Seconds)	Concen-tration (ug/L)
1. Aluminum			13. Magnesium		
2. Antimony			14. Manganese		
3. Arsenic			15. Mercury		
4. Barium			16. Nickel		
5. Beryllium			17. Potassium		
6. Cadmium			18. Selenium		
7. Calcium			19. Silver		
8. Chromium			20. Sodium		
9. Cobalt			21. Thallium		
10. Copper			22. Vanadium		
11. Iron			23. Zinc		
12. Lead					

Footnotes: • Indicate elements not analyzed by ICP with the notation "NA".

COMMENTS: _____

_____

_____

_____

Lab Manager _____

EXHIBIT D

ANALYTICAL METHODS

Analytical Methods

Any analytical method specified in Exhibit D may be utilized as long as the documented instrument or method detection limits meet the contract required detection levels (Exhibit C). Analytical methods with higher detection limits may be used only if the sample concentration exceeds twice the documented detection limit of the instrument or method. When an analyte concentration exceeds the calibrated or linear range, reanalysis of the prepared sample is required after appropriate dilution. All samples must initially be run undiluted (i.e. final product of sample preparation procedures). Both diluted and undiluted sample measurements must be contained in the raw data.

Labware must be acid cleaned according to EPA's manual "Methods for Chemical Analysis of Water and Wastes" (2) or an equivalent procedure. Samples must be opened and digested in a hood. Stock solutions for standards may be purchased or made up as specified in the analytical methods. Deionized water (Type II) is equivalent to distilled water. All sample dilutions should be made with acidified water to maintain constant acid strength. Background corrections are required for flame AA measurements below 350 nm and all furnace AA measurements. Before water sample preparation is initiated, check the pH of all water samples and note in the sample preparation log if it is less than 2 for metals and greater than 12 for cyanide.

NOTE: Reference numbers appear in parentheses throughout this section. See the last page of this exhibit for reference list.

1. Furnace AA/ICP/Flame AA - Water

Furnace Atomic Absorption

A. Sample Preparation (Furnace Digestion Procedure for Waters)

Shake sample and transfer 100 mL of well-mixed sample to a 250 mL beaker, add 1 mL of (1+1) HNO_3 and 2 mL 30% H_2O_2. Cover with watch glass or similar cover, heat for 2 hours at 95°C or until the volume is reduced to between 25 and 50 mL (make certain samples do not boil). Cool sample and filter (see Note 1) to remove insoluble material and bring back to 100 mL with deionized distilled water. The sample is now ready for analysis.

Concentrations so determined shall be reported as "total".

If Sb is to be determined by furnace, use the digestates prepared for ICP/AA analysis.

B. Analytical Procedures

See Attachment 1 (3).

Each furnace analysis requires at least 2 burns, except when performing full method of standard additions (MSA).

Inductively Coupled Plasma (ICP) and Flame Atomic Absorption (AA)

A. Sample Preparation (ICP/Flame AA Digestion Procedure for Water)

Shake sample and transfer 100 mL of a well mixed sample to a beaker. Add 2 mL of (1+1) HNO_3 and 10 mL of (1+1) HCl to the sample. Cover with watch glass or similar cover and heat on a steam bath or hot plate until the volume has been reduced to between 25 and 50 mL making certain the sample does not boil. After this treatment, cool sample and filter to remove insoluble material that could clog the nebulizer. (See Note 1.) Adjust the volume to 100 mL with deionized distilled water. The sample is now ready for analysis.

Concentrations so determined shall be reported as "total."

NOTE 1: In place of filtering, the sample after dilution and mixing may be centrifuged or allowed to settle by gravity overnight to remove insoluble material.

B. Analytical Procedures

ICP - See Attachment 3.
Flame AA - See Attachment 4.

2. Furnace AA/ICP/Flame AA - Sediment, Sludges and Soils

A. Sample Preparation - Acid Digestion of Sediments, Sludges and Soils

See Attachment 1.

B. Analytical Procedures

Furnace AA - See Attachment 2.
Each furnace analysis requires at least 2 burns, except for full MSA.
ICP - See Attachment 3.
Flame AA - See Attachment 4.

3. Mercury in Water
See Attachment 5 (manual) and 5A (automated).

4. Mercury in Sediment
See Attachment 6.

5. Cyanide in Water
See Attachment 7.

6. Cyanide in Sediment
See Attachment 8.

7. Determination of Percent Solids
See Attachment 9.

ATTACHMENT 1

SAMPLE PREPARATION OF SEDIMENTS, SLUDGES AND SOILS

1. Scope and Application

1.1 This method is an acid digestion procedure used to prepare sediments, sludges, and soil samples for analysis by flame or furnace atomic absorption spectroscopy (AAS) or by inductively coupled argon plasma spectroscopy (ICP). Samples prepared by this method may be analyzed by AAS or ICP for the following metals:

Aluminum	Chromium	Potassium
Antimony	Cobalt	Selenium
Arsenic	Copper	Silver
Barium	Iron	Sodium
Beryllium	Lead	Thallium
Cadmium	Magnesium	Vanadium
Calcium	Manganese	Zinc
	Nickel	

2. Summary of Method

NOTE: A separate digestion procedure is required for furnace AA and ICP analysis.

2.1 A representative 1 g (wet weight) sample is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed with either nitric acid or hydrochloric acid. Hydrochloric acid is used as the final reflux acid for the furnace AA analysis of Sb, the flame AA or ICP analysis of Al, Sb, Ba, Be, Ca, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Ni, K, Ag, Na, Tl, V and Zn. Nitric acid is employed as the final reflux acid for the furnace AA analysis of As, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Ag, Tl, V, and Zn. A separate sample shall be dried for a total solids determination (Exhibit D, Attachment 9).

3. Apparatus and Materials

3.1 250 ml beaker or other appropriate vessel.

3.2 Watch glasses

3.3 Thermometer that covers range of 0° to 200°C

3.4 Whatman No. 42 filter paper or equivalent

4. Reagents

4.1 ASTM Type II water (ASTM D1193): Water must be monitored.

4.2 Concentrated Nitric Acid (sp. gr. 1.41)

4.3 Concentrated Hydrochloric Acid (sp. gr. 1.19)

4.4 Hydrogen Peroxide (30%)

5. Sample Preservation, and Handling

5.1 Non-aqueous samples must be refrigerated upon receipt until analysis.

6. Procedure

6.1 Mix the sample thoroughly to achieve homogeniety. For each digestion procedure, weigh (to the nearest 0.01gms) a 1.0 to 1.5 gm portion of sample and transfer to a beaker.

6.2 Add 10 ml of 1:1 nitric acid (HNO_3), mix the slurry, and cover with a watch glass. Heat the sample to 95°C and reflux for 10 minutes without boiling. Allow the sample to cool, add 5 ml of concentrated HNO_3, replace the watch glass, and reflux for 30 minutes. Do not allow the volume to be reduced to less than 5 ml while maintaining a covering of solution over the bottom of the beaker.

6.3 After the second reflux step has been completed and the sample has cooled, add 2 ml of Type II water and 3 ml of 30% hydrogen peroxide (H_2O_2). Return the beaker to the hot plate for warming to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides, and cool the beaker.

6.4 Continue to add 30% H_2O_2 in 1 ml aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged. (NOTE: Do not add more than a total of 10 ml 30% H_2O_2.)

6.5 If the sample is being prepared for the furnace AA analysis of Sb, the flame AA or ICP analysis of Al, Sb, Ba, Be, Ca, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Ni, K, Ag, Na, Tl, V, and Zn, add 5 ml of 1:1 HCl and 10 ml of Type II water, return the covered beaker to the hot plate, and heat for an additional 10 minutes. After cooling, filter through Whatman No. 42 filter paper (or equivalent) and dilute to 100 ml with Type II water (or centrifuge the sample - see Note 1). The diluted sample has an approximate acid concentration of 2.5% (v/v) HCl and 5% (v/v) HNO_3. Dilute the digestate 1:1 (200 ml final volume) with the deionized water. The sample is now ready for analysis.

6.6 If the sample is being prepared for the furnace analysis of As, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Ag, Tl, V, and Zn, continue heating the acid-peroxide digestate until the volume has been reduced to approximately 2 ml, add 10 ml of Type II water, and warm the mixture After cooling, filter through Whatman No. 42 filter paper (or equivalent - see Note 1) and dilute to 100 ml with Type II water (or centrifuge the sample). The diluted digestate solution contains

approximately 2% (v/v) HNO_3. Dilute the digestate 1:1 (200 mL final volume) with deionized water. For analysis, withdraw aliquots of appropriate volume, and add any required reagent or matrix modifier. The sample is now ready for analysis.

7. Calculations

7.1 A separate determination of percent solids must be performed (Exhibit D, Attachment 9).

7.2 The concentrations determined in the digest are to be reported on the basis of the dry weight of the sample.

$$\text{Concentration (dry wt.) (mg/kg)} = \frac{C \times V}{W \times S}$$

where C = Concentration (mg/L)
V = Final volume in liters after sample preparation
W = Weight in kg of wet sample
S = % Solids/100

REF: Modification of Method 3050 from SW-846, Test Methods for Evaluating Solid Waste, EPA Office of Solid Waste and Emergency Response, July 1982.

8. Bibliography

1. Modification (by committee) of Method 3050, SW-846, 2nd ed., Test Methods for Evaluating Solid Waste, EPA Office of Solid Waste and Emergency Response, July 1982.

ATTACHMENT 2

ANTIMONY

Method 204.2 CLP-M (Atomic Absorption, furnace technique)

Optimum Concentration Range: 20-300 ug/l
Approximate Detection Limit: 3 ug/l

Preparation of Standard Solution

1. Stock solution: Carefully weigh 2.7426 g of antimony potassium tartrate (analytical reagent grade) and dissolve in deionized distilled water. Dilute to 1 liter with deionized water. 1 mL = 1 mg Sb (1000 mg/L).

2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. These solutions are also to be used for "standard additions".

3. The calibration standards must be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed after sample preparation.

Instrument Parameters (General)

1. Drying Time and Temp: 30 sec-125°C.
2. Ashing Time and Temp: 30 sec-800°C.
3. Atomizing Time and Temp: 10 sec-2700°C.
4. Purge Gas Atmosphere: Argon
5. Wavelength: 217.6 nm
6. Other operating parameters should be set as specified by the particular instrument manufacturer.

Notes

1. The above concentration values and instrument conditions are for a Perkin-Elmer HGA-2100, based on the use of a 20 ul injection, continuous flow purge gas and non-pyrolytic graphite and are to be used as guidelines only. Smaller size furnace devices or those employing faster rates of atomization can be operated using lower atomization temperatures for shorter time periods than the above recommended settings.

2. The use of background correction is required.

3. Nitrogen may also be used as the purge gas.

4. If chloride concentration presents a matrix problem or causes a loss previous to atomization, add an excess 5 mg of ammonium nitrate to the furnace and ash using a ramp accessory or with incremental steps until the recommended ashing temperature is reached.

5. For every sample analyzed, verification is necessary to determine that method of standard addition is not required (see Exhibit E).

6. If method of standard addition is required follow the procedure given in Exhibit E.

ARSENIC

Method 206.2 CLP-M (Atomic Absorption, furnace technique)

Optimum Concentration Range: 5-100 ug/l
Approximate Detection Limit: 1 ug/l

Preparation of Standard Solution

1. Stock solution: Dissolve 1.320 g of arsenic trioxide, As_2O_3 (analytical reagent grade) in 100 ml of deionized distilled water containing 4 g NaOH. Acidify the solution with 20 ml conc. HNO_3 and dilute to 1 liter. 1 ml = 1 mg As (1000 mg/l).
2. Nickel Nitrate Solution, 5%: Dissolve 24.780 g of ACS reagent grade $Ni(NO_3)_2 \cdot 6H_2O$ in deionized distilled water and make up to 100 ml.
3. Nickel Nitrate Solution, 1%: Dilute 20 ml of the 5% nickel nitrate to 100 ml with deionized distilled water.
4. Working Arsenic Solution: Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. Withdraw appropriate aliquots of the stock solution, add 1 ml of conc. HNO_3, 2 ml of 30% H_2O_2 and 2 ml of the 5% nickel nitrate solution. Dilute to 100 ml with deionized distilled water.

Sample Preparation

1. Add 100 ul of the 5% nickel nitrate solution to 5 ml of the digested sample. The sample is now ready for injection into the furnace.

Instrument Parameters (General)

1. Drying Time and Temp: 30 sec-125°C.
2. Ashing Time and Temp: 30 sec-1100°C.
3. Atomizing Time and Temp: 10 sec-2700°C.
4. Purge Gas Atmosphere: Argon
5. Wavelength: 193.7 nm
6. Other operating parameters should be set as specified by the particular instrument manufacturer.

Notes

1. The above concentration values and instrument conditions are for a Perkin-Elmer HGA-2100, based on the use of a 20 ul injection, purge gas interrupt and non-pyrolytic graphite. Smaller size furnace devices or those employing faster rates of atomization can be operated using lower atomization temperatures for shorter time periods than the above recommended settings.

2. The use of background correction is required.

3. For every sample analyzed, verification is necessary to determine that method of standard addition is not required (see Exhibit E).

4. If method of standard addition is required, follow the procedure given in Exhibit E).

5. The use of the Electrodeless Discharge Lamps (EDL) for the light source is recommended.

CADMIUM

Method 213.2 CLP-M (Atomic Absorption, furnace technique)

Optimum Concentration Range: 0.5-10 ug/l
Approximate Detection Limit: 0.1 ug/l

Preparation of Standard Solution

1. Stock solution: Carefully weigh 2.282g of cadmium sulfate, 3 Cd $SO_4 \cdot 8 H_2O$ (analytical reagent grade) and dissolve in deionized distilled water. Make up to 1 liter with deionized distilled water. 1 mL = 1 mg Cd (1000 mg/L).

2. Ammonium Phosphate solution (40%): Dissolve 40 grams of ammonium phosphate, $(NH_4)2HPO_4$ (analytical reagent grade) in deionized distilled water and dilute to 100 ml.

3. Prepare dilutions of stock cadmium solution to be used as calibration standards at the time of analysis. To each 100 ml of standard and sample alike add 2.0 ml of the ammonium phosphate solution. The calibration standards must be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed after sample preparation.

Instrument Parameters (General)

1. Drying Time and Temp: 30 sec-125°C.
2. Ashing Time and Temp: 30 sec-500°C.
3. Atomizing Time and Temp: 10 sec-1900°C.
4. Purge Gas Atmosphere: Argon
5. Wavelength: 228.8 nm
6. The operating parameters should be set as specified by the particular instrument manufacturer.

Notes

1. The above concentration values and instrument conditions are for a Perkin-Elmer HGA-2100, based on the use of a 20 ul injection, continuous flow purge gas and non-pyrolytic graphite and are to be used as guidelines only. Smaller size furnace devices or those employing faster rates of atomization can be operated using lower atomization temperatures for shorter time periods than the above recommended settings.

2. The use of background correction is required.

3. Contamination from the work area is critical in cadmium analysis. Use pipet tips which are free of cadmium.

4. For every sample analyzed, verification is necessary to determine that method of standard addition is not required (see Exhibit E).

5. If method of standard addition is required, follow the procedure given in Exhibit E.

LEAD

Method 239.2 CLP-M (Atomic Absorption, furnace technique)

Optimum Concentration Range: 5-100 ug/l
Approximate Detection Limit: 1 ug/l

Preparation of Standard Solution

1. Stock solution: Carefully weigh 1.599 g of lead nitrate, $Pb(NO_3)_2$ (analytical reagent grade), and dissolve in deionized distilled water. When solution is complete, acidify with 10 mL redistilled HNO_3 and dilute to 1 liter with deionized distilled water. 1 mL = 1 mg Pb (1000mg/L).

2. Lanthanum Nitrate solution: Dissolve 58.64 g of ACS reagent grade La_2O_3 in 100 ml conc. HNO_3 and dilute to 1000 ml with deionized distilled water. 1 ml = 50 mg La.

3. Working Lead solution: Prepare dilutions of stock lead solution to be used as calibration standards at the time of analysis. The calibration standards must be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed after sample preparation. To each 100 ml of diluted standard add 10 ml of the lanthanum nitrate solution.

Sample Preparation

1. To each 100 ml of prepared sample solution add 10 ml of the lanthanum nitrate solution.

Instrument Parameters (General)

1. Drying Time and Temp: 30 sec-125°C.

2. Ashing Time and Temp: 30 sec-500°C.

3. Atomizing Time and Temp: 10 sec-2700°C.
4. Purge Gas Atmosphere: Argon

5. Wavelength: 283.3 nm

6. Other operating parameters should be set as specified by the particular instrument manufacturer.

Notes

1. The above concentration values and instrument conditions are for a Perkin-Elmer HGA-2100, based on the use of a 20 ul injection, continuous flow purge gas and non-pyrolytic graphite and are to be used as guidelines only. Smaller size furnace devices or those employing faster rates of atomization can be operated using lower atomization temperatures for shorter time periods than the above recommended settings.

2. The use of background correction is required.

3. Greater sensitivity can be achieved using the 217.0 nm line, but the optimum concentration range is reduced. The use of a lead electrodeless discharge lamp at this lower wavelength has been found to be advantageous. Also a lower atomization temperature (2400°C) may be preferred.

4. To suppress sulfate interference (up to 1500 ppm) lanthanum is added as the nitrate to both samples and calibration standards. (Atomic Absorption Newsletter Vol. 15, No. 3, p. 71, May-June 1976).

5. Since glassware contamination is a severe problem in lead analysis, all glassware should be cleaned immediately prior to use, and once cleaned, should not be open to the atmosphere except when necessary.

6. For every sample analyzed, verification is necessary to determine that method of standard addition is not required (see Exhibit E).

7. If method of standard addition is required, follow the procedure given in Exhibit E.

SILVER

Method 272.2 CLP-M (Atomic Absorption, furnace technique)

Optimum Concentration Range: 1-25 ug/l
Approximate Detection Limit: 0.2 ug/l

Preparation of Standard Solution

1. Stock solution: Dissolve 1.575 g of $AgNO_3$ (analytical reagent grade) in deionized distilled water. Add 10 mL of concentrated HNO_3 and make up to 1 liter. 1 mL = 1 mg Ag (1000 mg/L).

2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. These solutions are also to be used for "standard additions".

3. The calibration standards must be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed after sample preparation.

Instrument Parameters (General)

1. Drying Time and Temp: 30 sec-125°C.
2. Ashing Time and Temp: 30 sec-400°C.
3. Atomizing Time and Temp: 10 sec-2700°C.
4. Purge Gas Atmosphere: Argon
5. Wavelength: 328.1 nm
6. Other operating parameters should be set as specified by the particular instrument manufacturer.

Notes

1. The above concentration values and instrument conditions are for a Perkin-Elmer HGA-2100, based on the use of a 20 ul injection, continuous flow purge gas and non-pyrolytic graphite and are to be used as guidelines only. Smaller size furnace devices or those employing faster rates of atomization can be operated using lower atomization temperatures for shorter time periods than the above recommended settings.

2. The use of background correction is required.

3. The use of halide acids should be avoided.

4. If absorption to container walls or formation of AgCl is suspected, see NOTE 3 under the Direct Aspiration Method (Exhibit D, Attachment 4).

5. For every sample analyzed, verification is necessary to determine that method of standard addition is not required (see Exhibit E).

6. If method of standard addition is required, follow the procedure given in Exhibit E.

ATTACHMENT 3

Method 200.7 CLP-M*
INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETRIC METHOD
FOR TRACE ELEMENT ANALYSIS OF WATER AND WASTES

1. Scope and Application

1.1 Dissolved elements are determined in filtered and acidified samples. Appropriate steps must be taken in all analyses to ensure that potential interferences are taken into account. This is especially true when dissolved solids exceed 1500 mg/L. (See 5.)

1.2 Total elements are determined after appropriate digestion procedures are performed. Since digestion techniques increase the dissolved solids content of the samples, appropriate steps must be taken to correct for potential interference effects. (See 5.)

1.3 Table 1 lists elements along with recommended wavelengths and typical estimated instrumental detection limits using conventional pneumatic nebulization. Actual working detected limits are sample dependent and as the sample matrix varies, these concentrations may also vary. In time, other elements may be added as more information becomes available and as required.

1.4 Because of the differences between various makes and models of satisfactory instruments, no detailed instrumental operating instructions can be provided. Instead, the analyst is referred to the instructions provided by the manufacturer of the particular instrument.

2. Summary of Method

2.1 The method describes a technique for the simultaneous or sequential multielement determination of trace elements in solution. The basis of the method is the measurement of atomic emission by an optical spectroscopic technique. Samples are nebulized and the aerosol that is produced is transported to the plasma torch where excitation occurs. Characteristic atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma (ICP). The spectra are dispersed by a grating spectrometer and the intensities of the line are monitored by photomultiplier tubes. The photocurrents from the photomultiplier tubes are processed and controlled by a computer system. A background correction technique is required to compensate for variable background contribution to the determination of trace elements. Background must be measured adjacent to analyte lines on

*CLP-M Modified for the Contract Laboratory Program

samples during analysis. The position selected for the background intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. The possibility of additional interferences named in 5.1 (and tests for their presence as described in 5.2) should also be recognized and appropriate corrections made.

3. Definitions

3.1 Dissolved — Those elements which will pass through a 0.45 um membrane filter.

3.2 Suspended — Those elements which are retained by a 0.45 um membrane filter.

3.3 Total — The concentration determined on an unfiltered sample following vigorous digestion.

3.4 Instrumental detection limits — See Exhibit E, pages 2 - 4.

3.5 Sensitivity — The slope of the analytical curve, i.e. functional relationship between emission intensity and concentration.

3.6 Instrument check standard — A multielement standard of known concentrations prepared by the analyst to monitor and verify instrument performance on a daily basis. (See 7.6.1.)

3.7 Interference check sample — A solution containing both interfering and analyte elements of known concentration that can be used to verify background and interelement correction factors. (See 7.6.2.)

3.8 Quality control sample — A solution obtained from an outside source having known concentration values to be used to verify the calibration standards. (See 7.6.3.)

3.9 Calibration standards — A series of known standard solutions used by the analyst for calibration of the instrument (i.e., preparation of the analytical curve). (See 7.4.)

3.10 Linear dynamic range — The concentration range over which the analytical curve remains linear as determined in Exhibit E.

3.11 Reagent blank — A volume of deionized, distilled water containing the same acid matrix as the calibration standards carried through the entire analytical scheme. (See 7.5.2.)

3.12 Calibration blank — A volume of deionized, distilled water acidified with HNO_3 and HCl. (See 7.5.1.)

3.13 Method of standard addition — The standard addition technique involves the use of the unknown and the unknown-plus-a-known amount of standard by adding known amounts of standard to one or more aliquots of the processed sample solution.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (11.7, 11.8 and 11.9) for the information of the analyst.

5. Interferences

5.1 Several types of interference effects may contribute to inaccuracies in the determination of trace elements. They can be summarized as follows:

5.1.1 Spectral interferences can be categorized as 1) overlap of a spectral line from another element; 2) unresolved overlap of molecular band spectra; 3) background contribution from continuous or recombination phenomena; and 4) background contribution from stray light from the line emission of high concentration elements. The first of these effects can be compensated by utilizing a computer correction of the raw data, requiring the monitoring and measurement of the interfering element. The second effect may require selection of an alternate wavelength. The third and fourth effects can usually be compensated by a background correction adjacent to the analyte line. In addition, users of simultaneous multi-element instrumentation must assume the responsibility of verifying the absence of spectral interference from an element that could occur in a sample but for which there is no channel in the instrument array. Listed in Table 2 are some interference effects for the recommended wavelengths given in Table 1. The data in Table 2 are intended for use only as a rudimentary guide for the indication of potential spectral interferences. For this purpose, linear relations between concentration and intensity for the analytes and the interferents can be assumed. The interference information, which was collected at the Ames Laboratory[1], is expressed as

[1]Ames Laboratory, USDOE, Iowa State University, Ames Iowa 50011

analyte concentration equivalents (i.e. false analyte concentrations) arising from 100 mg/L of the interferent element. The suggested use of this information is as follows: Assume that arsenic (at 193.696 nm) is to be determined in a sample containing approximately 10 mg/L of of aluminum. According to Table 2, 100 mg/L of aluminum would yield a false signal for arsenic equivalent to approximately 1.3 mg/L. Therefore, 10 mg/L of aluminum would result in a false signal for arsenic equivalent to approximately 0.13 mg/L. The reader is cautioned that other analytical systems may exhibit somewhat different levels of interference than those shown in Table 2, and that the interference effects must be evaluated for each individual system. Only those interferents listed were investigated and the blank spaces in Table 2 indicate that measurable interferences were not observed from the interferent concentrations listed in Table 3. Generally, interferences were discernible if they produced peaks or background shifts corresponding to 2-5% of the peaks generated by the analyte concentrations also listed in Table 3.

At present, information on the listed silver and potassium wavelengths are not available but it has been reported that second order energy from the magnesium 383.231 nm wavelength interferes with the listed potassium line at 766.491 nm.

5.1.2 Physical interferences are generally considered to be effects associated with the sample nebulization and transport processes. Such properties as change in viscosity and surface tension can cause significant inaccuracies especially in samples which may contain high dissolved solids and/or acid concentrations. The use of a peristaltic pump may lessen these interferences. If these types of interferences are operative, they must be reduced by dilution of the sample and/ or utilization of standard addition techniques. Another problem which can occur from high dissolved solids is salt buildup at the tip of the nebulizer. This affects aerosol flow rate causing instrumental drift. Wetting the argon prior to nebulization, the use of a tip washer, or sample dilution have been used to control this problem. Also, it has been reported that better control of the argon flow rate improves instrument performance. This is accomplished with the use of mass flow controllers.

5.1.3 Chemical interferences are characterized by molecular compound formation, ionization effects and solute vaporization effects. Normally these effects are not pronounced with the ICP technique, however, if observed they can be minimized by careful selection of operating conditions (that is, incident power, observation position, and so forth), by buffering of the sample, by matrix matching, and by standard addition procedures. These types of interferences can be highly dependent on matrix type and the specific analyte element.

5.2 For each group of samples of a similar matrix type and concentration (i.e., low, medium) for each Case of samples, or for each 20 samples received, whichever is more frequent, the following tests must be performed prior to reporting concentration data for analyte elements.

5.2.1 Serial dilution — If the analyte concentration is sufficiently high (minimally a factor of 10 above the instrument detection limit after dilution), an analysis of a 1:4 dilution must agree within 10 percent of the original determination. Serial dilution results must be reported on QC Report Form IX. Samples identified as Field Blanks cannot be used for serial dilution analysis.

If the dilution analysis is not within 10%, a chemical or physical interference effect should be suspected, and the data must be flagged with an "E".

6. Apparatus

6.1 Inductively Coupled Plasma-Atomic Emission Spectrometer.

6.1.1 Computer controlled atomic emission spectrometer with background correction.

6.1.2 Radiofrequency generator.

6.1.3 Argon gas supply, welding grade or better.

6.2 Operating conditions — Because of the differences between various makes and models of satisfactory instruments, no detailed operating instructions can be provided. Instead, the analyst should follow the instructions provided by the manufacturer of the particular instrument. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be investigated and established for each individual analyte line on that particular instrument. All measurements must be within the instrument linear range where correction factors are valid. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions used satisfy the analytical requirements and to maintain quality control data confirming instrument performance and analytical results.

7. Reagents and standards

7.1 Acids used in the preparation of standards and for sample processing must be ultra-high purity grade or equivalent. Redistilled acids are acceptable.

7.1.1 Acetic acid, conc. (sp gr 1.06).

7.1.2 Hydrochloric acid, conc. (sp gr 1.19).

7.1.3 Hydrochloric acid, (1+1): Add 500 mL conc. HCl (sp gr 1.19) to 400 mL deionized, distilled water and dilute to 1 liter.

7.1.4 Nitric acid, conc. (sp gr 1.41).

7.1.5 Nitric acid, (1+1): Add 500 mL conc. HNO_3 (sp gr 1.41) to 400 mL deionized, distilled water and dilute to 1 liter.

7.2 Deionized, distilled water: Prepare by passing distilled water through a mixed bed of cation and anion exchange resins. Use deionized, distilled water for the preparation of all reagents, calibration standards and as dilution water. The purity of this water must be equivalent to ASTM Type II reagent water of Specification D 1193 (14.6).

7.3 Standard stock solutions may be purchased or prepared from ultra high purity grade chemicals or metals. All salts must be dried for 1 h at 105° unless otherwise specified.

(CAUTION: Many metal salts are extremely toxic and may be fatal if swallowed. Wash hands thoroughly after handling.) Typical stock solution preparation procedures follow:

7.3.1 Aluminum solution, stock, 1 mL = 100 ug Al: Dissolved 0.100 g of aluminum metal in an acid mixture of 4 mL of (1+1) HCl and 1 mL of conc. HNO_3 in a beaker. Warm gently to effect solution. When solution is complete, transfer quantitatively to a liter flask, add an additional 10 mL of (1+1) HCl and dilute to 1000 mL with deionized, distilled water.

7.3.2 Antimony solution stock, 1 mL = 100 ug Sb: Dissolve 0.2669 g $K(SbO)C_4H_4O_6$ in deionized distilled water, add 10 mL (1+1) HCl and dilute to 1000 mL with deionized, distilled water.

7.3.3 Arsenic solution, stock, 1 mL = 100 ug As: Dissolve 0.1320 g of As_2O_3 in 100 mL of deionized, distilled water containing 0.4 g NaOH. Acidify the solution with 2 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.4 Barium solution, stock, 1 mL = 100 ug Ba: Dissolve 0.1516 g $BaCl_2$ (dried at 250°C for 2 hrs) in 10 mL deionized, distilled water with 1 mL (1+1) HCl. Add 10.0 mL (1+1) HCl and dilute to 1,000 mL with deionized, distilled water.

7.3.5 Beryllium solution, stock, 1 mL = 100 ug Be: Do not dry. Dissolve 1.966 g $BeSO_4 \cdot 4H_2O$, in deionized, distilled water, add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.6 Boron solution, stock, 1 mL = 100 ug B: Do not dry. Dissolve 0.5716 g anhydrous H_3BO_3 in deionized, distilled water and dilute to 1,000 mL. Use a reagent meeting ACS specifications keep the bottle tightly stoppered and store in a desiccator to prevent the entrance of atmospheric moisture.

7.3.7 Cadmium solution, stock, 1 mL = 100 ug Cd: Dissolve 0.1142 g CdO in a minimum amount of (1+1) HNO_3. Heat to increase rate of dissolution. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.8 Calcium solution, stock, 1 mL = 100 ug Ca: Suspend 0.2498 g $CaCO_3$ dried at 180°C for 1 h before weighing in deionized, distilled water and dissolve cautiously with a minimum amount of (1+1) HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.9 Chromium solution, stock, 1 mL = 100 ug Cr: Dissolve 0.1923 g of CrO_3 in deionized, distilled water. When solution is complete acidify with 10 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.10 Cobalt solution stock, 1 mL = 10 ug Co: Dissolve 0.1000 g of cobalt metal in a minimum amount of (1+1) HNO_3. Add 10.0 mL (1+1) HCl and dilute to 1,000 mL with deionized, distilled water.

7.3.11 Copper solution, stock, 1 mL = 100 ug Cu: Dissolve 0.1252 g CuO in a minimum amount of (1+1) HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.12 Iron solution, stock, 1 mL = 100 ug Fe: Dissolve 0.1430 g Fe_2O_3 in a warm mixture of 20 mL (1+1) HCl and 2 mL of conc. HNO_3. Cool, add an additional 5 mL of conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.13 Lead solution, stock, 1 mL = 100 ug Pb: Dissolve 0.1599 g $Pb(NO_3)_2$ in a minimum amount of (1+1) HNO_3. Add 10.0 mL of conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.14 Magnesium solution, stock, 1 mL = 100 ug Mg: Dissolve 0.1658 g MgO in a minimum amount of (1+1) HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.15 Manganese solution, stock, 1 mL = 100 ug Mn: Dissolve 0.1000 g of manganese metal in the acid mixture, 10 mL conc. HCl and 1 mL conc. HNO_3, and dilute to 1,000 mL with deionized, distilled water.

7.3.16 Molybdenum solution, stock, 1 mL = 100 ug Mo: Dissolve 0.2043 g $(NH_4)_2MoO_4$ in deionized, distilled water and dilute to 1,000 mL.

7.3.17 Nickel solution, stock, 1 mL = 100 ug Ni: Dissolve 0.1000 g of nickel metal in 10 mL hot conc. HNO_3, cool and dilute to 1,000 mL with deionized, distilled water.

7.3.18 Potassium solution, stock, 1 mL = 100 ug K: Dissolve 0.1907 g KCl, dried at 110°C, in deionized, distilled water. Dilute to 1,000 mL.

7.3.19 Selenium solution, stock, 1 mL = 100 ug Se: Do not dry. Dissolve 0.1727 g H_2SeO_3 (actual assay 94.6%) in deionized, distilled water and dilute to 1,000 mL.

7.3.20 Silica solution, stock, 1 mL = 100 ug SiO_2: Do not dry. Dissolve 0.4730 g $Na_2SiO_3 \cdot 9H_2O$ in deionized, distilled water. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.21 Silver solution, stock, 1 mL = 100 ug Ag: Dissolve 0.1575 g $AgNO_3$ in 100 mL of deionized, distilled water and 10 mL conc. HNO_3. Dilute to 1,000 mL with deionized, distilled water.

7.3.22 Sodium solution, stock, 1 mL = 100 ug Na: Dissolve 0.2542 g NaCl in deionized, distilled water. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.23 Thallium solution, stock, 1 mL = 100 ug Tl: Dissolve 0.1303 g $TlNO_3$ in deionized, distilled water. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.24 Vanadium solution, stock, 1 mL = 100 ug V: Dissolve 0.2297 NH_4VO_3 in a minimum amount of conc. HNO_3. Heat to increase rate of dissolution. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.25 Zinc solution, stock, 1 mL = 100 ug Zn: Dissolve 0.1245 g ZnO in a minimum amount of dilute HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.4 Mixed calibration standard solutions — Prepare mixed calibration standard solutions by combining appropriate volumes of the stock solutions in volumetric flasks. (See 7.4.1 thru 7.4.5.) Add 2 mL of (1+1) HNO_3 and 10 mL of (1+1) HCl and dilute to 100 mL with deionized, distilled water. (See Notes 1 and 6.) Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interference or the presence of impurities. Care should be taken when preparing the mixed standards that the elements are compatible and stable. Transfer the mixed standard solutions to a FEP fluorocarbon or unused polyethylene bottle for storage. Fresh mixed standards should be prepared as needed with the realization that concentration

can change on aging. Calibration standards must be initially verified using a quality control sample and monitored weekly for stability (see 7.6.3). Although not specifically required, some typical calibration standard combinations follow when using those specific wavelengths listed in Table 1.

7.4.1 Mixed standard solution I — Manganese, beryllium, cadmium, lead, and zinc.

7.4.2 Mixed standard solution II — Barium, copper, iron, vanadium, and cobalt.

7.4.3 Mixed standard solution III — Molybdenum, silica, arsenic, and selenium.

7.4.4 Mixed standard solution IV — Calcium, sodium, potassium, aluminum, chromium and nickel.

7.4.5 Mixed standard solution V — Antimony, boron, magnesium, silver, and thallium.

NOTE 1: If the addition of silver to the recommended acid combination results in an initial precipitation add 15 mL of deionized distilled water and warm the flask until the solution clears. Cool and dilute to 100 mL with deionized, distilled water. For this acid combination the silver concentration should be limited to 2 mg/L. Silver under these conditions is stable in a tap water matrix for 30 days. Higher concentrations of silver require additional HCl.

7.5 Two types of blanks are required for the analysis. The calibration blank (3.13) is used in establishing the analytical curve while the reagent blank (preparation blank, 3.12) is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

7.5.1 The calibration blank is prepared by diluting 2 mL of (1+1) HNO_3 and 10 mL of (1+1) HCl to 100 mL with deionized, distilled water. (See Note 6.) Prepare a sufficient quantity to be used to flush the system between standards and samples.

7.5.2 The reagent blank (or preparation blank - See Exhibit E) must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

7.6 In addition the calibration standards, an instrument check standard (3.6), an interference check sample (3.7) and a quality control sample (3.8) are also required for the analyses.

7.6.1 The instrument check standard for continuing calibration verification is prepared by the analyst by combining compatible elements at a concentration equivalent to the midpoint of their respective calibration curves. (See 10.1.3.)

7.6.2 The interference check sample is prepared by the analyst, or obtained from EPA if available (Exhibit E).

7.6.3 The quality control sample for the initial calibration verification should be prepared in the same acid matrix as the calibration standards and in accordance with the instructions provided by the supplier. EPA will either supply a quality control sample or information where one of equal quality can be procured. (See 10.1.1.)

8. Procedure

8.1 Set up instrument with proper operating parameters established in Section 6.2. The instrument must be allowed to become thermally stable before beginning. This usually requires at least 30 min. of operation prior to calibration.

8.2 Initiate appropriate operating configuration of computer.

8.3 Profile and calibrate instrument according to instrument manufacturer's recommended procedures, using mixed calibration standard solutions such as those described in Section 7.4. Flush the system with the calibration blank (7.5.1) between each standard. (See NOTE 7.) (Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.)

NOTE 7: For boron concentrations greater than 500 ug/L extended flush times of 1 to 2 minutes may be required.

8.4 Begin the sample run flushing the system with the calibration blank solution (7.5.1) between each sample. (See NOTE 7.) Analyze the instrument check standard (7.6.1) and the calibration blank (7.5.1) each 10 samples.

9. Calculation

9.1 Reagent blanks (preparation blanks) should be treated as specified in Exhibit E.

9.2 If dilutions were performed, the appropriate factor must be applied to sample values.

9.3 Data must be reported in ug/L.

10. Quality Control (Instrumental)

10.1 Check the instrument standardization by analyzing appropriate quality control check standards as follows:

10.1.1 A quality control sample (7.6.3) must be used daily for the initial calibration verification (See Exhibit E). A fresh dilution of this sample shall be analyzed every week thereafter to monitor their stability. If the results are not within ±10% of the true value listed for the control sample, prepare a new calibration standard and recalibrate the instrument. If this does not correct the problem, prepare a new stock standard and a new calibration standard and repeat the calibration.

10.1.2 Analyze the calibration blank (7.5.1) at a frequency of 10%. The result should be within ± contract required detection levels (Exhibit C). If the result is not within the control level, terminate the analysis, correct the problem and recalibrate the instrument (See Exhibit E).

10.1.3 For continuing calibration verification, analyze an appropriate instrument check standard (7.6.1) containing the elements of interest at a frequency of 10%. This check standard is used to determine instrument drift. If agreement is not within ±10% of the expected values, the analysis is out of control. The analysis must be terminated, the problem corrected, the instrument recalibrated, and the preceding 10 samples reanalyzed (See Exhibit E).

10.1.4 To verify interelement and background correction factors analyze the ICP interference check sample (7.6.2) at the beginning, and end of the sample run or a mimimum of twice per 8 hour work shift whichever is more frequent. The check sample must be analyzed initially at least 5 times repetitively to establish a mean value and standard deviation. Results must fall within the established control limits. If not, terminate the analysis, correct the problem, recalibrate the instrument, and reanalyze the samples (See Exhibit E).

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TABLE 1 - RECOMMENDED WAVELENGTHS(2) AND ESTIMATED INSTRUMENTAL DETECTION LIMITS

Element	Wavelength, nm(1)	Estimated Detection Limit, ug/L(2)
Aluminum	308.215	45
Antimony	206.833	32
Arsenic	193.696	53
Barium	455.403	2
Beryllium	313.042	0.3
Boron	249.773	5
Cadmium	226.502	4
Calcium	317.933	10
Chromium	267.716	7
Cobalt	228.616	7
Copper	324.754	6
Iron	259.940	7
Lead	220.353	42
Magnesium	279.079	30
Manganese	257.610	2
Molybdenum	202.030	8
Nickel	231.604	15
Potassium	766.491	see(3)
Selenium	196.026	75
Silica (SiO_2)	288.158	58
Silver	328.068	7
Sodium	588.995	29
Thallium	190.864	40
Vanadium	292.402	8
Zinc	213.856	2

(1) The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelength may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference. (See 5.1.1). The use of alternate wavelengths must be reported (in nm) with the sample data.

(2) The estimated instrumental detection limits as shown are taken from "Inductively Coupled Plasma-Atomic Emission Spectroscopy-Prominent Lines," EPA-600/4-79-017. They are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

(3) Highly dependent on operating conditions and plasma position.

TABLE 2. EXAMPLE OF ANALYTE CONCENTRATION EQUIVALENTS (mg/L) ARISING FROM INTERFERENTS AT THE 100 mg/L LEVEL

Analyte	Wavelength, nm	Interferent									
		Al	Ca	Cr	Cu	Fe	Mg	Mn	Ni	Ti	V
Aluminum	308.215	--	--	--	--	--	--	0.21	--	--	1.4
Antimony	206.833	0.47	--	2.9	--	0.08	--	--	--	.25	0.45
Arsenic	193.696	1.3	--	0.44	--	--	--	--	--	--	1.1
Barium	455.403	--	--	--	--	--	--	--	--	--	--
Beryllium	313.042	--	--	--	--	--	--	--	--	0.04	0.05
Boron	249.773	0.04	--	--	--	0.32	--	--	--	--	--
Cadmium	226.502	--	--	--	--	0.03	--	--	0.02	--	--
Calcium	317.933	--	--	0.08	--	0.01	0.01	0.04	--	0.03	0.03
Chromium	267.716	--	--	--	--	0.003	--	0.04	--	--	0.04
Cobalt	228.616	--	--	0.03	--	0.005	--	--	0.03	0.15	--
Copper	324.754	--	--	--	--	0.003	--	--	--	0.05	0.02
Iron	259.940	--	--	--	--	--	--	0.12	--	--	--
Lead	220.353	0.17	--	--	--	--	--	--	--	--	--
Magnesium	279.079	--	0.02	0.11	--	0.13	--	0.25	--	0.07	0.12
Manganese	257.610	0.005	--	0.01	--	0.002	0.002	--	--	--	--
Molybdenum	202.030	0.05	--	--	--	0.03	--	--	--	--	--
Nickel	231.604	--	--	--	--	--	--	--	--	--	--
Selenium	196.026	0.23	--	--	--	0.09	--	--	--	--	--
Silicon	288.158	--	--	0.07	--	--	--	--	--	--	0.01
Sodium	588.995	--	--	--	--	--	--	--	--	0.08	--
Thallium	190.864	0.30	--	--	--	--	--	--	--	--	--
Vanadium	292.402	--	--	0.05	--	0.005	--	--	--	0.02	--
Zinc	213.856	--	--	--	0.14	--	--	--	0.29	--	--

TABLE 3. INTERFERENT AND ANALYTE ELEMENTAL CONCENTRATIONS USED FOR INTERFERENCE MEASUREMENTS IN TABLE 2 (EXHIBIT D)

Analytes	(mg/L)	Interferents	(mg/L)
Al	10	Al	1000
As	10	Ca	1000
B	10	Cr	200
Ba	1	Cu	200
Be	1	Fe	1000
Ca	1	Mg	1000
Cd	10	Mn	200
Co	1	Ni	200
Cr	1	Ti	200
Cu	1	V	200
Fe	1		
Mg	1		
Mn	1		
Mo	10		
Na	10		
Ni	10		
Pb	10		
Sb	10		
Se	10		
Si	1		
Tl	10		
V	1		
Zn	10		

ATTACHMENT 5

MERCURY

Method 245.1 CLP-M* (Manual Cold Vapor Technique)

1. Scope and Application

1.1 In addition to inorganic forms of mercury, organic mercurials may also be present. These organo-mercury compounds will not respond to the cold vapor atomic absorption technique unless they are first broken down and converted to mercuric ions. Potassium permanganate oxidizes many of these compounds, but recent studies have shown that a number of organic mercurials, including phenyl mercuric acetate and methyl mercuric chloride, are only partially oxidized by this reagent. Potassium persulfate has been found to give approximately 100% recovery when used as the oxidant with these compounds. Therefore, a persulfate oxidation step following the addition of the permanganate has been included to insure that organo-mercury compounds, if present, will be oxidized to the mercuric ion before measurement. A heat step is required for methyl mercuric chloride when present in or spiked to a natural system. For distilled water the heat step is not necessary.

1.2 The range of the method may be varied through instrument and/or recorder expansion. Using a 100 ml sample, a detection limit of 0.2 ug Hg/l can be achieved (see Appendix 11.2).

2. Summary of Method

2.1 The flameless AA procedure is a physical method based on the absorption of radiation at 253.7 nm by mercury vapor. Organic mercury compounds are oxidized and the mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance (peak height) is measured as a function of mercury concentration and recorded in the usual manner.

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*CLP-M modified for the Contract Laboratory Program

3. Sample Handling and Preservation

3.1 Until more conclusive data are obtained, samples should be preserved by acidification with nitric acid to a pH of 2 or lower immediately at the time of collection.

4. Interference

4.1 Possible interference from sulfide is eliminated by the addition of potassium permanganate. Concentrations as high as 20 mg/l of sulfide as sodium sulfide do not interfere with the recovery of added inorganic mercury from distilled water.

4.2 Copper has also been reported to interfere; however, copper concentrations as high as 10 mg/l had no effect on recovery of mercury from spiked samples.

4.3 Sea waters, brines and industrial effluents high in chlorides require additional permanganate (as much as 25 ml). During the oxidation step, chlorides are converted to free chlorine which will also absorb radiation of 253 nm. Care must be taken to assure that free chlorine is absent before the mercury is reduced and swept into the cell. This may be accomplished by using an excess of hydroxylamine sulfate reagent (25 ml). In addition, the dead air space in the BOD bottle must be purged before the addition of stannous sulfate. Both inorganic and organic mercury spikes have been quantitatively recovered from the sea water using this technique.

4.4 Interference from certain volatile organic materials which will absorb at this wavelength is also possible. A preliminary run without reagents should determine if this type of interference is present (see Appendix 11.1).

5. Apparatus

5.1 Atomic Absorption Spectrophotometer: (See Note 1) Any atomic absorption unit having an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed. NOTE 1: Instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.

5.2 Mercury Hollow Cathode Lamp: Westinghouse WL-22847, argon filled, or equivalent.

5.3 Recorder: Any multi-range variable speed recorder that is compatible with the UV detection system is suitable.

5.4 Absorption Cell: Standard spectrophotometer cells 10 cm long, having quartz end windows may be used. Suitable cells may be constructed from plexiglass tubing, 1" O.D. X 4-1/2". The ends are ground perpendicular to the longitudinal axis and quartz windows (1" diameter X 1/16" thickness) are cemented in place. The cell is strapped to a burner for support and aligned in the light beam by use of two 2" by 2" cards. One inch diameter holes are cut in the middle of each card; the cards are then placed over each end of the cell. The cell is then positioned and adjusted vertically and horizontally to find the maximum transmittance.

5.5 Air Pump: Any peristaltic pump capable of delivering 1 liter of air per minute may be used. A Masterflex pump with electronic speed control has been found to be satisfactory.

5.6 Flowmeter: Capable of measuring an air flow of 1 liter per minute.

5.7 Aeration Tubing: A straight glass fit having a coarse porosity. Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return.

5.8 Drying Tube: 6" X 3/4" diameter tube containing 20 g of magnesium perchlorate (see Note 2). The apparatus is assembled as shown in Figure 1.

NOTE 2: In place of the magnesium perchlorate drying tube, a small reading lamp with 60W bulb may be used to prevent condensation of moisture inside the cell. The lamp is positioned to shine on the absorption cell maintaining the air temperature in the cell about 10°C above ambient.

6. <u>Reagents</u>

6.1 Sulfuric Acid, Conc: Reagent grade.

6.1.1 Sulfuric acid, 0.5 N: Dilute 14.0 ml of conc. sulfuric acid to 1.0 liter.

6.2 Nitric Acid, Conc: Reagent grade of low mercury content (see Note 3). NOTE 3: If a high reagent blank is obtained, it may be necessary to distill the nitric acid.

6.3 Stannous Sulfate: Add 25 g stannous sulfate to 250 ml of 0.5 N sulfuric acid. This mixture is a suspension and should be stirred continuously during use. (Stannous chloride may be used in place of stannous sulfate.)

6.4 Sodium Chloride-Hyroxylamine Sulfate Solution: Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in distilled water and dilute to 100 ml. (Hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate.)

6.5 Potassium Permanganate: 5% solution, w/v. Dissolve 5 g of potassium permanganate in 100 ml of distilled water.

6.6 Potassium Persulfate: 5% solution, w/v. Dissolve 5 g of potassium persulfate in 100 ml of distilled water.

6.7 Stock Mercury Solution: Dissolve 0.1354 g of mercuric chloride in 75 ml of distilled water. Add 10 ml of conc. nitric acid and adjust the volume to 100.0 ml. 1 ml = 1 mg Hg.

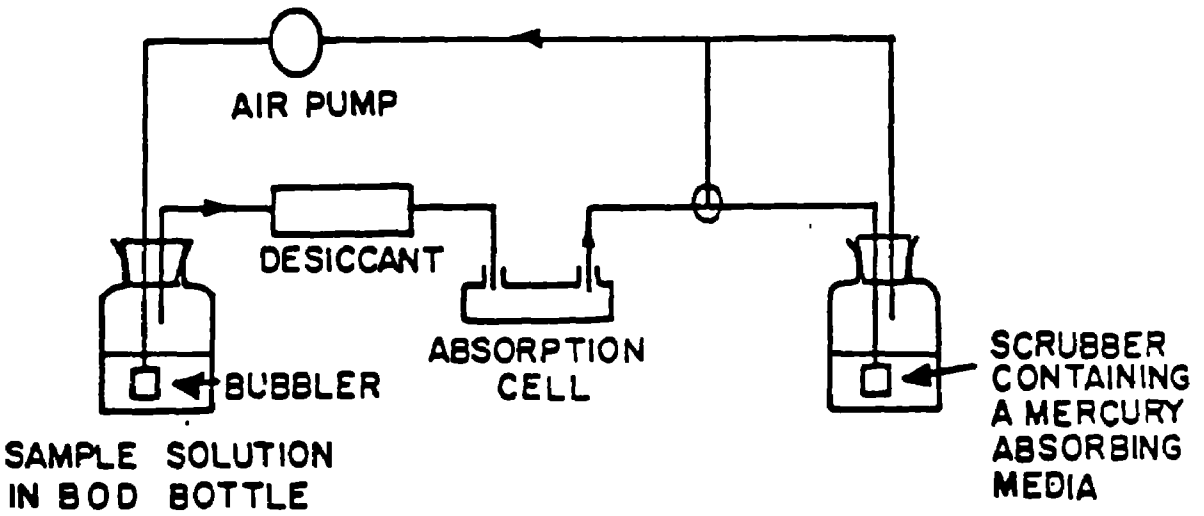

Figure 1. Apparatus for Flameless Mercury Determination.

6.8 Working Mercury Solution: Make successive dilutions of the stock mercury solution to obtain a working standard containing 0.1 ug per ml. This working standard and the dilutions of the stock mercury solution should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot.

7. Calibration

7.1 Transfer 0, 0.5, 1.0, 5.0 and 10.0 ml aliquots of the working mercury solution containing 0 to 1.0 ug of mercury to a series of 300 ml BOD bottles. Add enough distilled water to each bottle to make a total volume of 100 ml. Mix thoroughly and add 5 ml of conc. sulfuric acid (6.1) and 2.5 ml of conc. nitric acid (6.2) to each bottle. Add 15 ml of $KMnO_4$ (6.5) solution to each bottle and allow to stand at least 15 minutes. Add 8 ml of potassium persulfate (6.6) to each bottle and heat for 2 hours in a water bath maintained at 95°C. Cool and add 6 ml of sodium chloride-hydroxylamine sulfate solution (6.4) to reduce the excess permanganate. When the solution has been decolorized wait 30 seconds, add 5 ml of the stannous sulfate solution (6.3) and immediately attach the bottle to the aeration apparatus forming a closed system. At this point the sample is allowed to stand quietly without manual agitation. The circulating pump, which has previously been adjusted to a rate of 1 liter per minute, is allowed to run continuously (see Note 4). The absorbance will increase and reach maximum within 30 seconds. As soon as the recorder pen levels off, approximately 1 minute, open the bypass valve and continue the aeration until the absorbance returns to its minimum value (see Note 5). Close the bypass valve, remove the stopper and frit from the BOD bottle and continue the aeration. Proceed with the standards and construct a standard curve by plotting peak height versus micrograms of mercury.

NOTE 4: An open system where the mercury vapor is passed through the absorption cell only once may be used instead of the closed system.

NOTE 5: Because of the toxic nature of mercury vapor precaution must be taken to avoid its inhalation. Therefore, a bypass has been included in the system to either vent the mercury vapor into an exhaust hood or pass the vapor through some absorbing media, such as:

a) equal volumes of 0.1 M $KMnO_4$, and 10% H_2SO_4
b) 0.25% iodine in a 3% a KI solution

A specially treated charcoal that will adsorb mercury vapor is also available from Barnebey and Cheney, E. 8th Ave. and N. Cassidy St., Columbus, Ohio 43219, Cat #580-13 or #580-22.

8. Procedure

8.1 Transfer 100 ml, or an aliquot diluted to 100 ml, containing not more than 1.0 ug of mercury, to a 300 ml BOD bottle. Add 5 ml of sulfuric acid (6.1) and 2.5 ml of conc. nitric acid (6.2) mixing after each additon. Add 15 ml of potassium permanganate solution (6.5) to each sample bottle (see Note 6). For sewage samples additional permanganate may be required. Shake and add additional portions of potassium permanganate solution, if necessary, until the purple color persists for at least 15 minutes. Add 8 ml of potassium persulfate (6.6) to each bottle and heat for 2 hours in a water bath at 95°C.

NOTE 6: The same amount of $KMnO_4$ added to the samples should be present in standards and blanks.

Cool and add 6 ml of sodium chloride-hydroxylamine sulfate (6.4) to reduce the excess permanganate (see Note 7). After a delay of at least 30 seconds add 5 ml of stannous sulfate (6.3) and immediately attach the bottle to the aeration apparatus. Continue as described under Calibration.

NOTE 7: Add reductant in 6 mL increments until $KMnO_4$ is completely reduced.

9. Calculation

9.1 Determine the peak height of the unknown from the chart and read the mercury value from the standard curve.

9.2 Calculate the mercury concentration in the sample by the formula:

$$\text{ug Hg/l} = \text{ug Hg in aliquot} \times \frac{1{,}000}{\text{volume of aliquot in ml}}$$

9.3 Report mercury concentrations as follows: Below 0.2 ug/l, 0.2U; between 0.2 and 10 ug/l, one decimal; above 10 ug/l, whole numbers.

10. Appendix

10.1 While the possibility of absorption from certain organic substances actually being present in the sample does exist, EMSL has not encountered such samples. This is mentioned only to caution the analyst of the possibility. A simple correction that may be used is as follows: If an interference has been found to be present (4.4), the sample should be analyzed both by using the regular procedure and again under oxidizing conditions only, that is without the reducing reagents. The true mercury value can then be obtained by subtracting the two values.

10.2 If additional sensitivity is required, a 200 ml sample with recorder expansion may be used provided the instrument does not produce undue noise. Using a Coleman MAS-50 with a drying tube if magnesium perchlorate and a variable recorder, 2 mv was set to read full scale. With these conditions, and distilled water solutions of mercuric chloride at concentrations of 0.15, 0.10, 0.05 and 0.025 ug/l the standard deviations were ±0.027, ±0.0006, ±0.01 and ±0.004. Percent recoveries at these levels were 107, 83, 84 and 96%, respectively.

10.3 Directions for the disposal of mercury-containing wastes are given in ASTM Standards, Part 31, "Water", p. 349, Method D3223 (1976).

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ATTACHMENT 5A

MERCURY

Method 245.2 CLP-M* (Automated Cold Vapor Technique)

1. Scope and Application

1.1 The working range is 0.2 to 20.0 ug Hg/l.

2. Summary of Method

2.1 The flameless AA procedure is a physical method based on the absorption of radiation at 253.7 nm by mercury vapor. The mercury is reduced to the elemental state and aerated from solution. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance (peak height) is measured as a function of mercury concentration and recorded in the usual manner.

2.2 In addition to inorganic forms of mercury, organic mercurials may also be present. These organo-mercury compounds will not respond to the flameless atomic absorption technique unless they are first broken down and converted to mercuric ions. Potassium permanganate oxidizes many of these compounds, but recent studies have shown that a number of organic mercurials, including phenyl mercuric acetate and methyl mercuric chloride, are only partially oxidized by this reagent. Potassium persulfate has been found to give approximately 100% recovery when used as the oxidant with these compounds. Therefore, an automated persulfate oxidation step following the automated addition of the permanganate has been included to insure that organo-mercury compounds, if present, will be oxidized to the mercuric ion before measurement.

3. Sample Handling and Preservation

3.1 Until more conclusive data are obtained, samples should be preserved by acidification with nitric acid to a pH of 2 or lower immediately at the time of collection[1] (see Exhibit F).

* CLP-M Modified for the contract Laboratory Program

4. Interference (see NOTE 1)

4.1 Some sea waters and waste-waters high in chlorides have shown a positive interference, probably due to the formation of free chlorine.

4.2 Interference from certain volatile organic materials which will absorb at this wavelength is also possible. A preliminary run under oxidizing conditions, without stannous sulfate, would determine if this type of interference is present.

4.3 Formation of a heavy precipitate, in some wastewaters and effluents, has been reported upon addition of concentrated sulfuric acid. If this is encountered, the problem sample cannot be analyzed by this method.

4.4 Samples containing solids must be blended and then mixed while being sampled if total mercury values are to be reported.

NOTE 1: All the above interferences can be overcome by use of the Manual Mercury method.

5. Apparatus

5.1 Technicon Auto Analyzer consisting of:

5.1.1 Sampler II with provision for sample mixing.

5.1.2 Manifold.

5.1.3 Proportioning Pump II or III.

5.1.4 High temperature heating bath with two distillation coils (Technicon Part #116-0163) in series.

5.2 Vapor-liquid separator (Figure 1).

5.3 Absorption cell, 100 mm long, 10 mm diameter with quartz windows.

5.4 Atomic Absorption Spectrophotometer (see Note 2): Any atomic absorption unit having an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed.

NOTE 2: Instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.

5.5 Mercury Hollow Cathode Lamp: Westinghouse WL-22847, argon filled, or equivalent.

5.6 Recorder: Any multi-range variable speed recorder that is compatible with the UV detection system is suitable.

5.7 Source of cooling water for jacketed mixing coil and connector A-7.

5.8 Heat lamp: A small reading lamp with 60W bulb may be used to prevent condensation of moisture inside the cell. The lamp is positioned to shine on the absorption cell maintaining the air temperature in the cell about 10°C above ambient.

6. Reagents

6.1 Sulfuric Acid, Conc: Reagent grade

6.1.1 Sulfuric acid, 2 N: Dilute 56 ml of conc. sulfuric acid to 1 liter with distilled water.

6.1.2 Sulfuric acid, 10%: Dilute 100 ml conc. sulfuric acid to 1 liter with distilled water.

6.2 Nitric acid, Conc: Reagent grade of low mercury content.

6.2.1 Nitric Acid, 0.5% Wash Solution: Dilute 5 ml of concentrated nitric acid to 1 liter with distilled water.

6.3 Stannous Sulfate: Add 50 g stannous sulfate to 500 ml of 2 N sulfuric acid (6.1.1). This mixture is a suspension and should be stirred continuously during use.

NOTE 3: Stannous chloride may be used in place of stannous sulfate.

6.4 Sodium Chloride-Hydroxylamine Sulfate Solution: Dissolve 30 g of sodium chloride and 30 g of hydroxylamine sulfate in distilled water to 1 liter.

NOTE 4: Hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate.

6.5 Potassium Permanganate: 0.5% solution, w/v. Dissolve 5 g of potassium permanganate in 1 liter of distilled water.

6.6 Potassium Permanganate, 0.1 N: Dissolve 3.16 g of potassium permanganate in distilled water and dilute to 1 liter.

6.7 Potassium Persulfate: 0.5% solution, w/v. Dissolve 5 g potassium persulfate in 1 liter of distilled water.

6.8 Stock Mercury Solution: Dissolve 0.1354 g of mercuric chloride in 75 ml of distilled water. Add 10 ml of conc. nitric acid and adjust the volume to 100.0 ml. 1.0 ml = 1.0 mg Hg.

6.9 Working Mercury Solution: Make successive dilutions of the stock mercury solution (6.8) to obtain a working standard containing 0.1 ug per ml. This working standard and the dilutions of the stock mercury solution should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot. From this solution prepare standards containing 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0 ug Hg/l.

6.10 Air Scrubber Solution: Mix equal volumes of 0.1 N potassium permanganate (6.6) and 10% sulfuric acid (6.1.2).

7. Procedure

7.1 Set up manifold as shown in Figure 2.

7.2 Feeding all the reagents through the system with acid wash solution (6.2.1) through the sample line, adjust heating bath to 105°C.

7.3 Turn on atomic absorption spectrophotometer, adjust instrument settings as recommended by the manufacturer, align absorption cell in light path for maximum transmittance and place heat lamp directly over absorption cell.

7.4 Arrange working mercury standards from 0.2 to 20.0 ug Hg/l in sampler and start sampling. Complete loading of sample tray with unknown samples.

7.5 Prepare standard curve by plotting peak height of processed standards against concentration values. Determine concentration of samples by comparing sample peak height with standard curve.

NOTE 5: Because of the toxic nature of mercury vapor, precaution must be taken to avoid its inhalation. Venting the mercury vapor into an exhaust hood or passing the vapor through some absorbing media such as:

a) equal volumes of 0.1 N $KMnO_4$(6.6) and 10% H_2SO_4 (6.1.2).
b) 0.25% iodine in a 3% KI solution, is recommended.

A specially treated charcoal that will adsorb mercury vapor is also availale from Barnebey and Cheney, E. 8th Ave. and North Cassidy St., Columbus, Ohio 43219, Cat, #580-13 or #580-22.

7.6 After the analysis is complete put all lines except the H_2SO_4 line in distilled water to wash out system. After flushing, was out the H_2SO_4 line. Also flush the coils in the high temperature heating bath by pumping stannous sulfate (6.3) through the sample lines followed by distilled water. This will prevent build-up of oxides of manganese.

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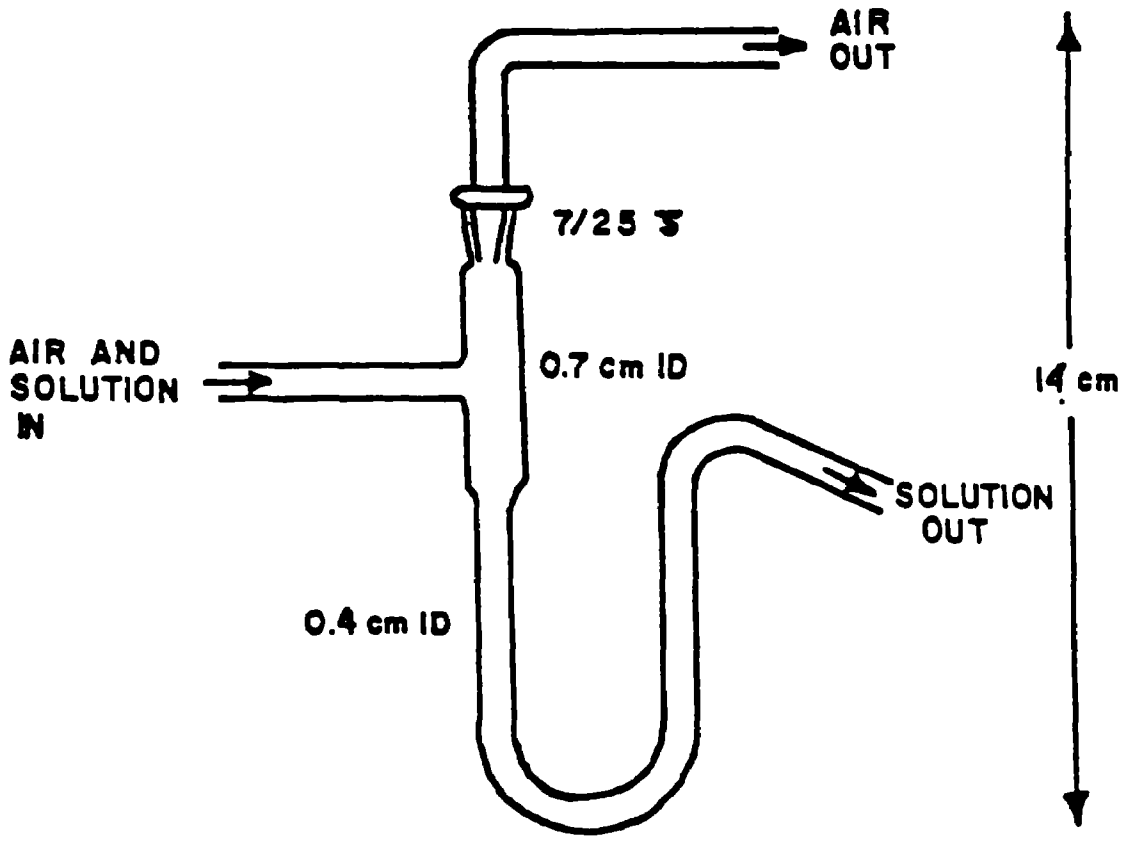

Figure 1. Vapor liquid separator.

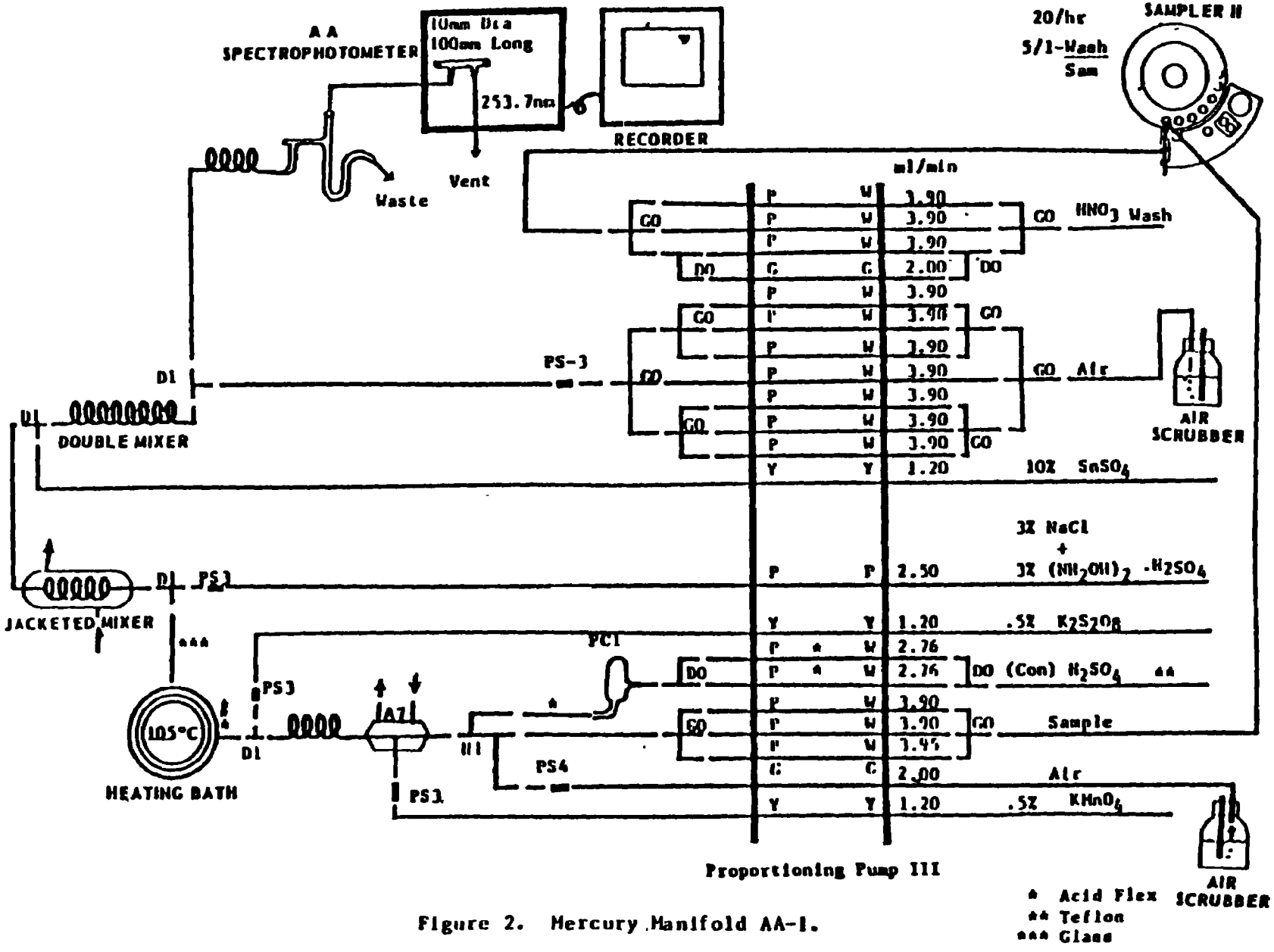

Figure 2. Mercury Manifold AA-I.

ATTACHMENT 6

MERCURY (in Sediments)

Method 245.5 CLP-M* (Manual Cold Vapor Technique)

1. Scope and Application

1.1 This procedure measures total mercury (organic and inorganic) in soils, sediments, bottom deposits and sludge type materials.

1.2 The range of the method is 0.2 to 5 ug/g. The range may be extended above or below the normal range by increasing or decreasing sample size or through instrument and recorder control.

2. Summary of Method

2.1 A weighed portion of the sample is acid digested for 2 minutes at 95°C, followed by oxidation with potassium permanganate and potassium persulfate. Mercury in the digested sample is then measured by the conventional cold vapor technique.

2.2 An alternate digestion involving the use of an autoclave is describe in (8.2).

3. Sample Handling and Preservation

3.1 Because of the extreme sensitivity of the analytical procedure and the omnipresence of mercury, care must be taken to avoid extraneous contamination. Sampling devices and sample containers should be ascertained to be free of mercury; the sample should not be exposed to any condition in the laboratory that may result in contact or air-borne mercury contamination.

3.2 Refrigerate solid samples upon receipt.

3.3 The sample should be analyzed without drying. A separate % solids determination is required. (Exhibit D, Attachment 9).

4. Interferences

4.1 The same types of interferences that may occur in water samples are also possible with sediments, i.e., sulfides, high copper, high chlorides, etc.

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*CLP-M modified for the Contract Laboratory Program

4.2 Volatile materials which absorb at 253.7 nm will cause a positive interference. In order to remove any interfering volatile materials, purge the dead air space in the BOD bottle before the addition of stannous sulfate.

4.3 Sample containing high concentrations of oxidizable organic materials, as evidenced by high chemical oxygen demand values, may not be completely oxidized by this procedure. When this occurs, the recovery of organic mercury will be low. The problem can be eliminated by reducing the weight of the original sample or by increasing the amount of potassium persulfate (and consequently stannous chloride) used in the digestion.

5. Apparatus

5.1 Atomic Absorption Spectrophotometer (see Note 1): Any atomic absorption unit having an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed.

NOTE 1: Instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.

5.2 Mercury Hollow Cathode Lamp: Westinghouse WL-22847, argon filled, or equivalent.

5.3 Recorder: Any multi-range variable speed recorder that is compatible with the UV detection system is suitable.

5.4 Absorption Cell: Standard spectrophotometer cells 10 cm long, having quartz end windows may be used. Suitable cells many be constructed from pexiglass tubing, 1" O.D. X 4-1/2". The ends are ground perpendicular to the longitudinal axis and quartz windows (1" diameter X 1/16" thickness) are cemented in place. Gas inlet and outlet ports (also of plexiglass but 1/4" O.D.) are attached approximately 1/2" from each end. The cell is strapped to a burner for support and aligned in the light beam to give the maximum transmittance.

NOTE 2: Two 2" X 2" cards with one inch diameter holes may be placed over each end of the cell to assist in positioning the cell for maximum transmittance.

5.5 Air Pump: Any peristaltic pump capable of delivering 1 liter or air per minute may be used. A Masterflex pump with electronic speed control has been found to be satisfatory. (Regulated compressed air can be used in an open one-pass system.)

5.6 Flowmeter: Capable of measuring an air flow of 1 liter per minute.

5.7 Aeration Tubing: Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return. Straight glass tubing terminating in a coarse porous frit is used for sparging air into the sample.

5.8 Drying Tube: 6" X 3/4" diameter tube containing 20 g of magnesium perchlorate (see Note 3). The apparatus is assembled as shown in the accompanying diagram.

NOTE 3: In place of the magnesium perchlorate during tube, a small reading lamp with 60W bulb may be used to prevent condensation of moisture inside the cell. The lamp is positioned to shine on the absorption cell maintaining the air temperature in the cell about 10°C above ambient.

6. Reagents

6.1 Sulfuric acid, conc.: Reagent grade of low mercury content.

6.2 Nitric acid, conc.: Reagent grade of low mercury content.

6.3 Stannous Sulfate: Add 25 g stannous sulfate to 250 ml of 0.5 N sulfuric acid (6.2). This mixture is a suspension and should be stirred continuously during use.

6.4 Sodium Chloride-Hydroxylamine Sulfate Solution: Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in distilled water and dilute to 100 ml.

NOTE 4: A 10% solution of stannous chloride may be substituted for (6.3) and hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate in (6.4).

6.5 Potassium Permanganate: 5% solution, w/v. Dissolve 5 g of potassium permanganate in 100 ml of distilled water.

6.6 Potassium Persulfate: 5% solution, w/v. Dissolve 5 g of potassium persulfate in 100 ml of distilled water.

6.7 Stock Mercury Solution: Dissolve 0.1354 g of mercuric chloride in 75 ml of distilled water. Add ml of conc. nitric acid and adjust the volume to 100.0 ml. 1.0 = 1.0 mg Hg.

6.8 Working Mercury Solution: Make successive dilutions of the stock mercury solution (6.7) to obtain a working standard containing 0.1 ug/ml. This working standard and the dilution of the stock mercury solutions should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot.

7. Calibration

7.1 Transfer 0, 0.5, 1.0, 5.0 and 10 ml aliquots of the working mercury solutions (6.8) containing 0 to 1.0 ug of mercury to a series of 300 ml BOD bottles. Add enough distilled water to each bottle to make a total volume of 10 ml. Add 5 ml of conc. H_2SO_4 (6.1) and 2.5 ml of conc. HNO_3 (6.2) and heat 2 minutes in a water bath at 95°C. Allow the sample to cool and add 50 ml distilled water, 15 ml of $KMnO_4$ solution (6.5) and 8 ml of potassium persulfate solution (6.6) to each bottle and return to the water bath for 30 minutes. Cool and add 6 ml of sodium chloride-hydroxylamine sulfate solution (6.4) to reduce the excess permanganate. Add 50 ml of distilled water. Treating each bottle individually, add 5 ml of stannous sulfate solution (6.3) and immediately attach the bottle to the aeration apparatus. At this point the sample is allowed to stand quietly without manual agitation. The circulating pump, which has previously been adjusted to a rate of 1 liter per minute, is allowed to run continuously. The absorbance, as exhibited either on the spectrophotometer or the recorder, will increase and reach maximum within 30 seconds. As soon as the recorder pen levels off, approximately 1 minute, open the bypass valve and continue the aeration until the absorbance returns to its minimum value (see Note 5). Close the bypass valve, remove the fritted tubing from the BOD bottle and continue the aeration. Proceed with the standards and construct a standard curve by plotting peak height versus micrograms of mercury.

NOTE 5: Because of the toxic nature of mercury vapor precaution must be taken to avoid its inhalation. Therefore, a bypass has been included in the system to either vent the mercury vapor into an exhaust hood or pass the vapor through some absorbing media, such as:

a) equal volumes of 0.1 N $KMnO_4$ and 10% H_2SO_4
b) 0.25% iodine in a 3% KI solution

A specially treated charcoal that will absorb mercury vapor is also available from Barnebey and Cheney, E. 8th Avenue and N. Cassidy Sreet, Columbus, Ohio 43219

8. Procedure

8.1 Weigh a representative 0.2 g portion of wet sample and place in the bottom of a BOD bottle. Add 5 mL of sulfuric acid (6.1) and 2.5 mL of concentrated nitric acid (6.2) mixing after each addition. Heat two minutes in a water bath at 95°C. Cool, add 50 ml distilled water, 15 mL potassium permanganate solution (6.5) and 8 mL of potassium persulfate solution (6.6) to each sample bottle. Mix thoroughly and place in the water bath for 30 minutes at 95°C. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate (6.4) to reduce the excess permanganate. Add 55 mL of distilled water. Treating each bottle individually, add 5 mL of stannous sulfate (6.3) and immediately attach the bottle to the aeration apparatus. Continue as described under (7.1).

8.2 An alternate digestion procedure employing an autoclave may also be used. In this method 5 ml of conc. H_2SO_4 and 2 ml of conc. HNO_3 are added to the 0.2 g of sample. 5 ml of saturated $KMnO_4$ solution and 8 ml of potassium persulfate solution are added and the bottle is covered with a piece of aluminum foil. The sample is autoclaved at 121°C and 15 lbs. for 15 minutes. Cool, make up to a volume of 100 ml with distilled water and add 6 ml of sodium chloride-hydroxylamine sulfate solution (6.4) to reduce the excess permanganate. Purge the dead air space and continue as described under (7.1).

9. Calculations

9.1 Measure the peak height of the unknown from the chart and read the mercury value from the standard curve.

9.2 Calculate the mercury concentration in the sample by the formula:

$$\text{ug Hg/g} = \frac{\text{ug Hg in the aliquot}}{\text{wt of the aliquot in gms (based upon dry wt of the sample)}}$$

9.3 Report mercury concentrations as follows: Below 0.1 ug/gm, 0.1U; between 0.1 and 1 ug/gm, to the nearest 0.01 ug; between 1 and 10 ug/gm, to nearest 0.1 ug; above 10 ug/gm, to nearest ug.

Bibliography

1. Bishop, J. N., "Mercury in Sediments", Ontario Water Resources Comm., Toronto, Ontario, Canada, 1971.

2. Salma, M., private communication, EPA Cal/Nev. Basin Office, Almeda, California.

3. "Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue," USEPA Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, August 1977, Revised October 1980.

4. Op. cit. (#3), Methods 245.1 or 245.2.

EXHIBIT E

QUALITY ASSURANCE/QUALITY CONTROL REQUIREMENTS

GENERAL QA/QC CONSIDERATIONS

Standard laboratory practices for laboratory cleanliness as applied to glassware and apparatus must be adhered to. Laboratory practices with regard to reagents, solvents, and gases should also be adhered to. For additional guidelines regarding these general laboratory procedures, please see Sections 4 and 5 of the Handbook for Analytical Quality Control in Water and Wastewater Laboratories EPA-600/4-79-019, USEPA Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, March 1979.

QUALITY ASSURANCE REQUIREMENTS

The quality assurance/quality control (QA/QC) procedures defined below must be used by the Contractor when performing the methods specified in Exhibit D. When additional QA/QC procedures are specified in the methods in Exhibit D, the Contractor must also follow these procedures. The cost of performing all QA/QC procedures specified in this Statement of Work is included in the price of performing the bid lot.

The purpose of this document is to provide a uniform set of procedures for the analysis of inorganic constituents of samples, documentation of methods and their performance during a survey, and verification of the sample data generated. The program will also assist laboratory personnel in recalling and defending their actions under cross examination if required to present court testimony in enforcement case litigation.

The prime function of the QA/QC program outlined here is the definition of procedures for the evaluation and documentation of sampling and analytical methodologies and the reduction and reporting of data. The objective is to provide a uniform basis for sample collection and handling, instrument and methods maintenance, performance evaluation, and analytical data gathering and reporting. Although it is impossible to address all analytical situations in one document, the approach taken here is to define minimum requirements for all major steps relevant to any inorganic analysis. In many instances where methodologies are available, specific quality control procedures are incorporated into the method documentation. Ideally, samples involved in enforcement actions are analyzed only after the methods have met the minimum performance and documentation requirements described in this document.

The Contractor must participate in the Laboratory Audit and Intercomparison Study Program run by EPA, EMSL-Las Vegas. The Contractor can expect to analyze two samples per three-month contract period for this program.

The Contractor must perform and report to SMO and EMSL/LV on Form XI quarterly verification of instrument detection limits (IDL) by the method specified in Exhibit E, by type and model for each instrument used on this contract. All the IDLs must meet the CRDLs specified in Exhibit C. For ICP methods, the Contractor must also report linearity range verification, all interelement correction factors, wavelengths used and integration times on a quarterly basis on Forms XII and XIII.

For QA/QC procedures, two different types of "samples" are specified. A "sample received" is the field or PE sample each of which has an EPA number. An "analytical sample" is each "analysis" performed (i.e. each cup, tube or container in autosamplers, etc). A "frequency of 10%" means once every 10 analytical samples.

The Contractor must report all QC data in the exact format shown in Forms II - XIII (See Exhibit B).

This section outlines the minimum QA/QC operations necessary to satisfy the analytical requirements of the contract. The following QA/QC operations must be performed as stated in this exhibit:

1. Initial Calibration and Calibration Verification
2. Continuing Calibration Verification
3. Preparation Blank Analysis
4. Interference Check Sample Analysis
5. ICP Serial Dilution Analysis
6. Matrix Spike Analysis
7. Duplicate Sample Analysis
8. Furnace AA QC Analysis (Method of Standard Additions may be required under certain conditions).
9. Laboratory Quality Control Sample Analysis

1. <u>Initial Calibration and Calibration Verification</u>

Guidelines for instrumental calibration are given in EPA 600/4-79-020 and/or Exhibit D. Instruments must be calibrated daily and each time the instrument is set up.

<u>Instrument Calibration</u>

For atomic absorption systems, calibration standards are prepared by diluting the stock metal solutions at the time of analysis. Low calibration standards must be prepared fresh each time an analysis is to be made and discarded after use. Prepare a blank and at least three calibration standards in graduated amounts in the appropriate range. One atomic absorption calibration standard must be at the CRDL except for mercury. The calibration standards must be prepared using the same type of acid or combination of acids and at the same concentration as will result in the samples following sample preparation. Beginning with the blank and working toward the highest standard, aspirate or inject the solutions and record the readings. Calibration standards for furnace procedures should be prepared as described in the individual methods for that metal.

For cyanide and mercury, follow the calibration procedures outlined in Exhibit D. One cyanide calibration standard must be at the CRDL. For ICP systems, calibrate the instrument according to instrument manufacturer's recommended procedures.

<u>Initial Calibration Verification Standards</u>

After the ICP, AA and cyanide systems have been calibrated, the accuracy of the initial calibration shall be verified and documented for every analyte by the analysis of EPA Initial Calibration Verification Solution.

The initial calibration verification standard must be run at each wavelength used for analysis.

If the Initial Calibration Verification Solution is not available from EPA, or where a certified solution of an analyte is not available from any source, analyses shall be conducted on an independent standard at a concentration other than that used for calibration, but within the calibration range. An independent standard is defined as a standard composed of the analytes from a different source than those used in the standards for the initial calibration. For ICP, the Initial Calibration Verification Solution must be run at each wavelength used in the analysis of the sample. When measurements exceed the control limits of Table 1, the analysis must be terminated, the problem corrected, the instrument recalibrated, and the calibration reverified.

The values for the initial and subsequent continuing calibration verificaions shall be recorded on Form II (see Exhibit B) for ICP, AA, and cyanide analyses, as indicated.

Instrument Detection Limit Determination

Before any field samples are analyzed under this contract, the instrumental detection limits (in ug/L) must be determined within 30 days of the start of the analyses and at least quarterly (every 3 months), and must meet the levels specified in Exhibit C. The instrumental detection limits (in ug/L) shall be determined by multiplying by 3, the average of the standard deviations obtained on three nonconsecutive days from the analysis of a standard solution (each analyte in reagent water) at a concentration 3-5 times IDL, with 7 consecutive measurements per day.

For each Case, IDLs must be reported on QC Report Form VII. If multiple AA instruments are used for the analysis of an element within a Case, the highest IDL for the AAs must be reported for that Case. Use the same reporting procedure for multiple ICPs.

Linear Range Analysis

To verify linearity near the CRDL for ICP analysis, the contractor must analyze an ICP standard at two times the CRDL at the beginning and end of each sample analysis run, or a minimum of twice per 8 hour working shift, whichever is more frequent. This standard must be run for all elements analyzed by ICP except Al, Ba, Ca, Fe, Mg, Na and K.

For ICP analysis, a linear range verification check standard must be analyzed and reported quarterly for each element on Form XII. The standard must be analyzed during a routine analytical run performed under this contract. The analytically determined concentration of this standard must be within $\pm$ 5% of the true value. This concentration is the upper limit of the ICP linear range beyond which results cannot be reported under this contract.

Calibration Blank

A calibration blank is analyzed each time the instrument is calibrated, at the beginning and the end of the run, and at a frequency of 10% during the run. The results for the calibration blank solution shall be recorded on Form III for ICP, AA and cyanide analyses, as indicated. Blanks are to be reported down to the instrument detection limit. If this blank result is greater than the CRDL (Exhibit C), terminate analysis, correct the problem and recalibrate.

2. Continuing Calibration Verification

To assure calibration accuracy during each analysis run, one of the following standards is to be used for continuing calibration verification and must be analyzed for each analyte at a frequency of 10% or every 2 hours during an analysis run, whichever is more frequent. The standard must also be analyzed for each analyte at the beginning of the run and after the last analytical sample. The analyte concentrations in the continuing calibration standard must be one of the following solutions at or near the mid-range levels of the calibration curve:

1. EPA Solutions

2. NBS SRM 1643a

3. A contractor-prepared standard solution

TABLE 1. INITIAL AND CONTINUING CALIBRATION VERIFICATION CONTROL LIMITS FOR INORGANIC ANALYSES

Analytical Method	Inorganic Species	% of True Value (EPA Set)	
		Low Limit	High Limit
ICP/AA	Metals	90	110
Cold Vapor AA	Mercury	80	120
Other	Cyanide	85	115

The same continuing calibration standard must be used throughout the analysis runs for a case of samples received.

If the deviation of the continuing calibration verification is greater than the Control Limits specified in Table 1, the instrument must be recalibrated and the preceding 10 samples reanalyzed for the analytes affected. Information regarding the continuing verification of calibration shall be recorded on Form II (see Exhibit B) for ICP, AA and cyanide as indicated.

3. Preparation Blank Analysis

At least one preparation blank (or reagent blank), consisting of deionized distilled water processed through each sample preparation procedure (i.e. water, solids) performed for each Case, must be prepared and analyzed with every 20 samples received, or with each batch* of samples digested whichever is more frequent (see Exhibit D). The first 20 samples of a Case are to be assigned to preparation blank one, and the second 20 samples to preparation blank two, etc. (see Form III). Each data package must contain the results of all the preparation blank analyses associated with the samples in that Case.

This blank is to be reported for each Case and used in all analyses to ascertain whether sample concentrations reflect contamination in the following manner:

1. If the concentration of the blank is less than or equal to the contract required detection level (Exhibit C), no correction of sample results is performed.

2. If the concentration of the blank is above the contract required detection level: For any group of samples associated with a particular blank, the concentration of the sample with the least concentrated analyte must be 10X the blank concentration, or all samples associated with the blank and less than 10 times the blank concentration must be redigested and reanalyzed, with the exception of an identified aqueous-soil field blank. The sample value is not to be corrected for the blank value.

   The values for the preparation blank shall be recorded in ug/L on Form III (see Exhibit B) for ICP, AA, and cyanide analyses, as indicated.

4. ICP Interference Check Sample Analysis

To verify inter-element and background correction factors the Contractor must analyze and report the results for an ICP Interference Check Sample at the beginning and end of each sample analysis run (or a minimum of twice per 8 hour working shift, whichever is most frequent). The ICP Interference Check Sample must be obtained from EPA (EMSL/LV) if available. Results for the check sample analysis during the analytical runs must fall within the control limit of ±20% of the true value for the analytes included in the Interference Check Sample. If not, terminate the analysis, correct the problem, recalibrate, reverify the calibration, and reanalyze the samples. If the ICP Interference Check Sample is not available from EPA, an independent ICP Check Sample must be prepared with interferent and analyte concentrations at the levels specified in Table 2 (Exhibit E). The mean value and standard deviation must be established by initially analyzing the check samples at least 5 times repetitively for each parameter on Form IV. Results must fall within the control limit of ±20% of the established mean value.

Results of all Interference Check Sample analyses for all ICP parameters must be recorded on Form IV (see Exhibit B).

*A group of samples prepared at the same time.

5. ICP Serial Dilution Analysis

Prior to reporting concentation data for the analyte elements, the Contractor must analyze and report the results of the ICP Serial Dilution Analysis. The ICP Serial Dilution Analysis must be performed on each group of samples of a similar matrix type (i.e. water, soil) and concentration (i.e. low, medium) for each Case of samples, or for each 20 samples received, whichever is more frequent. Samples identified as field blanks cannot be used for serial dilution analysis. If the analyte concentration is sufficiently high (minimally a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:4 dilution must agree within 10 percent of the original determination. If the dilution analysis is not within 10%, a chemical or physical interference effect should be suspected, and the data must be flagged with an "E." Serial dilution results must be reported on QC Report Form IX.

6. Spiked Sample Analysis

The spiked sample analysis is designed to provide information about the effect of the sample matrix on the digestion and measurement methodology. The spike is added before the digestion and prior to any distillation steps (i.e., CN^-). At least one spiked sample analysis must be performed on each group of samples of a similar matrix type (i.e. water, soil) and concentration (i.e. low, medium) for each Case of samples or for each 20 samples received, whichever is more frequent.* Samples identified as field blanks cannot be used for spiked sample analysis. The analyte spike must be added in the amount given in Table 3 (Exhibit E) for each element analyzed. If two analytical methods are used to obtain the reported values for the same element for a Case of samples (i.e. ICP, GFAA), spike samples must be run by each method used. If the spike recovery is not within the limits of 75-125%, the data of all samples received associated with that spiked sample must be flagged with the letter "N". An exception to this rule is granted in situations where the sample concentration exceeds the spike concentration by a factor of four or more. In such a case, the spike recovery should not be considered and the data shall be reported unflagged even if the percent recovery does not meet the 75-125% recovery criteria. In the instance where there is more than one spiked sample per matrix per Case, if one spike sample recovery is not within contract criteria, flag all the samples of the same matrix in the Case. Individual component percent recoveries (%R) are calculated as follows:

$$\%\text{Recovery} = \frac{(SSR-SR)}{SA} \times 100$$

Where SSR = Spiked Sample Result
SR = Sample Result
SA = Spike Added

When sample concentration is less than the instrument detection limit, use SR = 0 for purposes of calculating %Recovery. The spiked sample results must be reported on Form V for ICP, AA and cyanide analyses, as indicated.

*EPA may require additional spike sample analysis, upon request, for which the contractor will be paid.

TABLE 2. INTERFERENT AND ANALYTE ELEMENTAL CONCENTRATIONS USED FOR ICP INTERFERENCE CHECK SAMPLE

Analytes	(mg/L)	Interferents	(mg/L)
Ba	0.5	Al	500
Be	0.5	Ca	500
Cd	1.0	Fe	200
Co	0.5	Mg	500
Cr	0.5		
Cu	0.5		
Mn	0.5		
Ni	1.0		
Pb	1.0		
V	0.5		
Zn	1.0		

TABLE 3. SPIKING LEVELS[1] FOR SPIKED SAMPLE ANALYSIS

Element	For ICP/AA (ug/L)		For Furnace AA (ug/L)		Other (ug/L)
	Water	Sediment[1]	Water	Sediment[1]	
Aluminum	2,000	*			
Antimony	500	500	100	100	
Arsenic			20	40	
Barium	2,000	2,000			
Beryllium	50	50			
Cadmium	50	50	5	5	
Calcium	*	*			
Chromium	200	200			
Cobalt	500	500			
Copper	250	250			
Iron	1,000	*			
Lead	500	500	20	50	
Magnesium	*	*			
Manganese	200	500			
Mercury					1
Nickel	400	500			
Potassium	*	*			
Selenium			10	10	
Silver	50	50			
Sodium	*	*			
Thallium			50	50	
Vanadium	500	500			
Zinc	200	500			
Cyanide					100

NOTE: Elements without spike levels and not designated with an asterisk, should be spiked at appropriate levels.

[1]The levels shown indicate concentrations in the final digestate of the spiked sample (200 mL FV)

*No spike required.

7. Duplicate Sample Analysis

At least one duplicate sample must be analyzed from each group of samples of a similar matrix type (i.e. water, soil) and concentration (i.e. low, medium) for each Case of samples or for each 20 samples received, whichever is more frequent.* Samples identified as field blanks cannot be used for duplicate sample analysis. If two analytical methods are used to obtain the reported values for the same element for a Case of samples (i.e. ICP, GFAA), duplicate samples must be run by each method used. The relative percent differences+ (RPD) for each component are calculated as follows:

$$RPD = \frac{|D_1 - D_2|}{(D_1+D_2)/2} \times 100$$

Where RPD = Relative Percent Difference
D_1 = First Sample Value
D_2 = Second Sample Value (duplicate)

The results of the duplicate sample analysis must be reported on Form VI. (See Exhibit B). A control limit of ± 20% for RPD shall be used for sample values greater than 5 times the contract required detection level (CRDL). A control limit of ± the CRDL shall be used for sample values less than 5 times the CRDL (Exhibit C), and this control limit (±CRDL) should be entered in the "Control Limit" column on Form VI. If one result is above the 5 x CRDL level and the other is below, use the ± CRDL criteria. If either sample value is less than the CRDL, the RPD is not calculated and is indicated as "NC" on Form VI.

If the duplicate sample results are outside the control limits, flag all the data for samples received associated with that duplicate sample with an "*" on Forms I and V. In the instance where there is more than one duplicate sample per Case, if one duplicate result is not within contract criteria, flag all the samples of the same matrix in the Case. The percent difference data will be used by EPA to evaluate the long-term precision of the methods for each parameter. Specific control limits for each element will be added to Form VI at a later date based on these precision results.

8. Furnace Atomic Absorption (AA) QC Analysis

Because of the nature of the Furnace AA technique, the special procedures summarized in Figure 1 will be required for quantitation. (These procedures are not meant to replace those included in Exhibit D of this document, but will supplement the guidance provided therein.)

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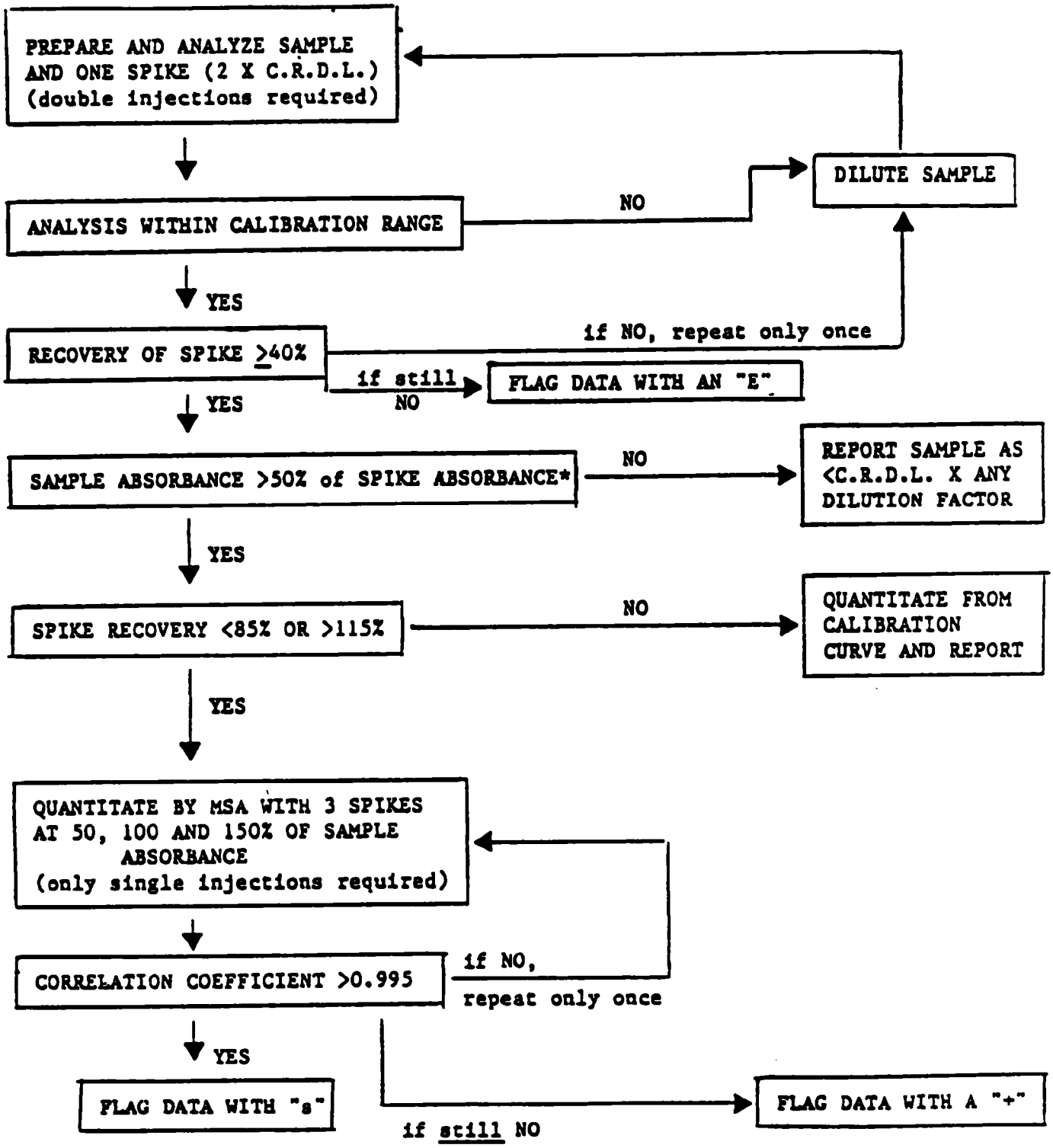
*EPA may require additional duplicates be analyzed, on request, for which the contractor will be paid.

+Relative percent difference is equivalent to relative range of duplicates (RR).

Figure 1.

FURNACE ATOMIC ABSORPTION ANALYSIS SCHEME



*spike absorbance defined as (absorbance of spike sample) minus (absorbance of the sample)

1. All furnace analyses, except during Full Methods of Standard Addition (MSA), will require duplicate injections for which the average absorbance or "concentration" will be reported. All analyses must fall within the calibration range. The raw data package must contain both absorbance or "concentration" values, the average value and the relative standard deviation (RSD) or coefficient of variation (CV). For concentrations greater than CRDL, the duplicate injection readings must agree within 20% RSD or CV, or the sample must be rerun once. If the readings are still out, flag the value with an "M" on Form I.

2. All furnace analyses for each sample will require at least a single analytical spike to determine if the MSA will be required for quantitation. Analytical spikes are not required on the predigest spike samples. The spike* will be required to be at a concentration (in the sample) twice the contract required detection limit (CRDL). The percent (%R) of the spike, calculated by the same formula as Spiked Sample analyses (Exhibit E), will then determine how the sample will be quantitated as follows:

   a) If the spike recovery is less than 40%, the sample must be diluted and rerun with another spike. Dilute the sample by a factor of 5 to 10 and rerun. This step must only be performed once. If after the dilution the spike recovery is still <40%, report data and flag with an "E" to indicate interference problems.

   b) If the spike recovery is greater than 40% and the sample absorbance or concentration is <50% of the spike+, report the sample as less than the CRDL or less than the CRDL times the dilution factor if the sample was diluted.

   c) If the sample absorbance or concentration is >50% of the spike+ and the spike recovery is between 85% and 115%, the sample should be quantitated directly from the calibration curve.

   d) If the sample absorbance or concentration is >50% of the spike+ and the spike recovery is less than 85% or greater than 115%, the sample must be quantitated by MSA.

3. The following procedures will be incorporated into MSA analyses.

   a) Data from MSA calculations must be within the linear range as determined by the calibration curve generated at the beginning of the analytical run.

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*Spikes are post digest spikes to be prepared prior to analysis by adding a known quantity of the analyte to an aliquot of the digested sample. The unspiked sample aliquot must be compensated for any volume change in the spike samples by addition of deionized water to the unspiked sample aliquot.

+"Spike" is defined as (absorbance or concentration of spike sample) minus (absorbance or concentration of the sample).

b) The sample and three spikes must be analyzed consecutively for MSA quantitation (the "initial" spike run data is specifically excluded from use in the MSA quantitation). Only single injections are required for MSA quantitation.

c) Spikes* should be prepared such that:

- Spike 1 is approximately 50% of the sample absorbance.
- Spike 2 is approximately 100% of the sample absorbance.
- Spike 3 is approximately 150% of the sample absorbance.

d) The data for each MSA analysis should be clearly identified in the raw data documentation along with the slope, intercept and correlation coefficient (r) for the least squares fit of the data and the results reported on Form VIII. Reported values obtained by MSA are flagged on the data sheet (Form I) with the letter "s".

e) If the correlation coefficient (r) for a particular analysis is less than 0.995 the MSA analyses must be repeated once. If the correlation coefficient is still <0.995, the results on Form I must be flagged with "+".

9. Laboratory Control Sample Analysis

Laboratory Control Sample (LCS) - Aqueous and solid laboratory quality control samples must be analyzed for each analyte using the same sample preparation and analytical methods employed for the EPA samples received.

The aqueous LCS solution must be obtained from EPA (if unavailable, the EPA Initial Calibration Verification solution may be used). The aqueous LCS must be prepared and analyzed with the samples for each of the procedures applied to each case of samples received. One aqueous LCS must be analyzed for every 20 samples received, or for each batch+ of samples digested whichever is more frequent (see Exhibit D). Each data package must contain the results of all the LCS analyses associated with the samples on that Case. For cyanide, the distilled mid-range calibration standard may be used as the aqueous LCS (see Section 8.3.2.1, Exhibit D). An aqueous LCS is not required for mercury analysis.

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*Spikes are post digest spikes to be prepared prior to analysis by adding a known quantity of the analyte to an aliquot of the digested sample. The unspiked sample aliquot must be compensated for any volume change in the spiked samples by addition of deionized water to the unspiked sample aliquot.

+A group of samples prepared at the same time.

All aqueous LCS results will be reported on Form VII in terms of true concentration and percent recovery (%R) as calculated by:

$$\%R = (\text{Observed}/\text{True}) \times 100$$

where "observed" is the measured concentration. If the % recovery for the aqueous LCS falls outside the control limits of 80% - 120%, the analyses must be terminated, the problems corrected and the previous samples associated with that LCS re-analyzed (ie., previous 19 samples or the batch of samples from the case).

Once a month, a solid LCS, available from EMSL-LV must be prepared and analyzed using each of the procedures applied to the solid samples received. If this EPA solid LCS is unavailable, other EPA Quality Assurance Check samples or other certified materials may be used.

The monthly results of the solid LCS samples should be reported on a duplicate Form VII and submitted monthly to EMSL/Las Vegas and SMO on the 15th of every month.

If the percent recovery for the solid LCS sample is outside the control limits established by EPA, no further sample analyses may be done until the analytical problems are solved, and satisfactory LCS results are obtained.

EXHIBIT G

CHAIN-OF-CUSTODY AND DOCUMENT CONTROL PROCEDURES

NOTES

The contractor shall not deviate from the procedures described herein without the prior written approval of the Contracting Officer: Provided, that the Contracting Officer may ratify in writing such deviation and such ratification shall constitute the approval required herein.

Specifications for Chain-of-Custody and Document Control Procedures

The contractor must have written standard operating procedures (SOP) for receipt of samples, maintenance of custody, tracking the analysis of samples and assembly of completed data and must follow these SOPs. These procedures are necessary to ensure that analytical data collected under this contract are acceptable for use in EPA enforcement case preparations and litigation. The Contractor's SOPs shall provide mechanisms and documentation to meet each of the following specifications and shall be used by EPA for the basis for laboratory evidence audits.

1. The contractor shall have a designated sample custodian responsible for receipt of the samples.

2. The contractor shall have written SOPs for receiving and logging in of the samples. The procedures shall include documentation of the sample condition, maintenance of custody and sample security and documentation of verification of sample tag information against custody records.

3. The contractor shall have written SOPs for maintenance of the security of the sample after log in and shall demonstrate security of the sample storage and laboratory areas.

4. The contractor shall have written SOPs for tracking the work performed on any particular sample. The tracking system shall include standard data logging formats, logbook entry procedures and a means of controlling logbook pages, computer printouts, chart tracing and other written or printed documents relevant to the samples. Logbooks, printed forms or other written documentation must be available to describe the work performed in each of the following stages of analysis:

   - Sample receipt
   - Sample preparation
   - Standard preparation/tracking
   - ICP analysis
   - Flame, flameless and cold vapor AA analysis
   - Non-metal Inorganics analysis
   - Data reduction
   - Data reporting

5. The contractor shall have written SOPs for organization and assembly of all documents relating to each EPA case. Documents shall be filed on a case specific basis. The procedures must ensure that all documents including logbook pages, sample tracking records, measurement readout records, computer printouts, raw data summaries, correspondence and any other written documents having reference to the case, are compiled in one location for submission to EPA in the Complete Case File Purge package (formerly termed the "Document Control and Chain-of-Custody Package.") The Case File Purge package must be compiled within 7 days of submission of the sample data package. The system must include a document numbering and inventory procedure.

6. The Complete Case File Purge package includes but is not limited to: sample tags, custody records, sample tracking records, analysts log-book pages, bench sheets, measurement readout records, extraction and analysis chronicles, computer printouts, raw data summaries, instrument logbook pages, correspondence, and the document inventory.

Hazardous Waste Disposal Site
Contract Analytical Support
Chain-of-Custody and Document Control Procedures

Sample Control

A sample is physical evidence collected from a facility or from the environment. An essential part of this investigations effort is the control of the evidence gathered. To accomplish this, the following chain-of-custody and document control procedures have been established.

Sample Identification

Each sample bottle shall be labeled with a tag containing the sample number and sample description to identify the contents of the bottle. Additionally, the sample number shall be marked on the outside of any special packaging container to facilitate identification. Typical sample tags are shown in Figure 1.

Chain-of-Custody Procedures

Because of the nature of the data being collected, the possession of samples must be traceable from the time the samples are collected until they are introduced as evidence in legal proceedings. To maintain and document sample custody, the chain-of-custody procedures described here are followed.

A sample is under custody if:

1. It is in your actual possession, or

2. It is in your view, after being in your physical possession, or

3. It was in your possession and then you locked or sealed it up to prevent tampering, or

4. It is in a secure area.

To assure custody of samples during transport and shipping, each sample within a packing container is recorded on a chain-of-custody record shown in Figure 2. Each sample number is recorded and the number of containers shipped is recorded on the sheets. Also record the other information regarding the project, samples (or shipper if returning empty bottles), method of shipment and remember to sign and date the sheet. The original custody sheet is then placed inside the package (protected from damage) and the package sealed. Sample containers, shipping boxes, coolers or other packages may be sealed by using the seal shown in Figure 3. The seal must be placed so the container cannot be opened without breaking the seal.

Upon receipt of samples in custody, inspect the package and note any damage to the sealing tape or custody seals. Note on the custody record or other logbook that the seals or locks were intact upon receipt if no tampering or damage appears to have occurred. Open the package and verify that each item listed on the sheet is present and correctly identified. If all data and samples are correct, sign and date the "received by Laboratory by" box. In the event errors are noted, record the discrepancies in the remarks column (initial and date each comment) then sign the chain-of-custody record. Report discrepancies to the Sample Management Office for remedies.

Laboratory Document Control

The goal of the Document Control Program is to assure that all documents for a specified case (group of samples) will be accounted for when the project is completed. The program includes a document numbering and inventory procedure for preparation of the specified documentation packages for each case.

Logbooks

All observations and results recorded by the laboratory but not on preprinted data sheets are entered into permanent laboratory logbooks. Data recorded are referenced with the case number, date and analyst's signature at the top of the page. Data from only one case are recorded per page. When all the data from a case is compiled, copies of all logbook entries must be included in the documentation package.

Instrument logs are also limited to one case per page with the case number recorded at the top of each page. Copies of these logs must also be included in the final documentation package.

Corrections to Documentation

All documentation in logbooks and other documents shall be in ink. If an error is made in a logbook assigned to one individual, that person should make corrections simply by crossing a line through the error and entering the correct information. Changes made subsequently are dated and initialed. Corrections made to other data records or non-personal logbooks are made by crossing a single line through the error, entering the correct information and initialing and dating the correction.

Consistency of Documentation

Before releasing analytical results, the laboratory assembles and cross checks the information on sample tags, custody records, lab bench sheets, personal and instrument logs andother relevant data to ensure that data pertaining to each particular sample or case is consistent throughout the record.

Document Numbering and Inventory Procedure

In order to provide document accountability of the completed analysis records, each item in a case is inventoried and assigned a serialized number and an identifier associating it to the case and Region.

Case # - Region - Serialized number

For example - 75-2-0240

All documents relevant to each case including: logbook pages, bench sheets, instrument readings, chart recordings, custody records, etc., are inventoried. Each data generator (analyst) is responsible for ensuring that all documents generated are placed in the file for inventory and returned to EPA. Figure 4 is an example of a document inventory.

Confidential Information

Any samples or information received with a request of confidentiality is handled as "confidential." A separate, locked file is maintained and segregated from other nonconfidential information. Data generated from confidential samples is treated as "confidential." Upon receipt of confidential information, the DCO* logs these documents into a Confidential Inventory Log. The information is then made available to authorized personnel, but only after it has been signed out to that person by the DCO. The documents shall be returned to the locked file at the conclusion of each working day. Confidential information may not be reproduced except upon approval by and under the supervision of the DCO. Any reproduction must be kept to an absolute minimum. The DCO will enter all copies into the document control system and apply the same requirements as the original. In addition, this information may not be destroyed except upon approval by and under the supervision of the EPA program manager and EPA contracting officer. The DCO shall remove and retain the cover page of any confidential information disposed of for one year and shall keep a record of the destruction in the Confidential Inventory Log.

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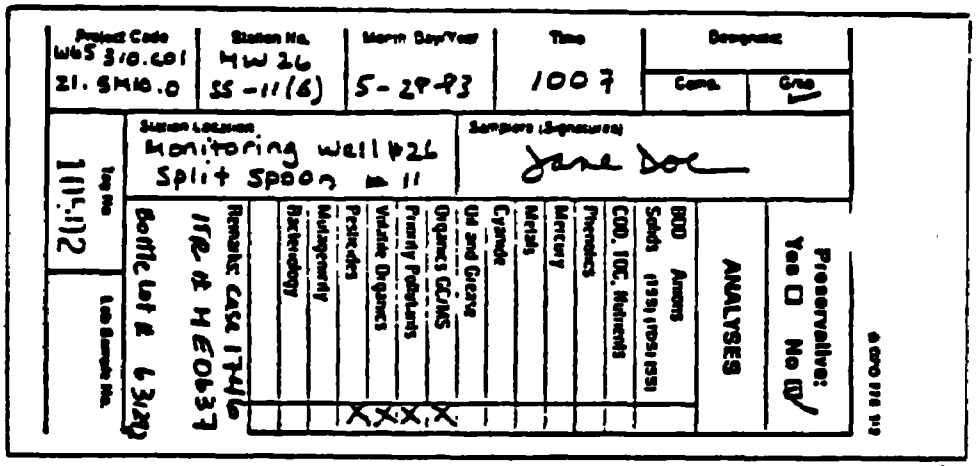
*DCO is a document control officer assigned by the contractor to maintain control of confidential information.



Project Code: WBS 310.601 21.SM10.0
Station No.: MW 26 SS-11(6)
Month Day/Year: 5-29-93
Time: 1007
Designate: Comp. / Grab ✓
Station Location: Monitoring well #26 Split Spoon # 11
Samplers (Signatures): Jane Doe
Tag No.: 111512
Lab Sample No.:
Preservative: Yes ☐ No ☑
ANALYSES
BOD Anions
Solids (TSS, TDS, TSS)
COD, TOC, Nutrients
Phenolics
Mercury
Metals
Cyanide
Oil and Grease
Organics GC/MS
Priority Pollutants
Volatile Organics
Pesticides
Radiography
Radioactivity
Mutagenicity
Remarks: CASE 17416 Site # HE0037 Bottle Lot # 63XQ
X X X X

EPA

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Figure 1. Example sample tag.

ENVIRONMENTAL PROTECTION AGENCY
Office of Enforcement

CHAIN OF CUSTODY RECORD

PROJ. NO.	PROJECT NAME					NO. OF CONTAINERS							REMARKS
SAMPLERS (Signature)													
STA. NO.	DATE	TIME	COMP.	GRAB	STATION LOCATION								

Relinquished by (Signature)	Date / Time	Received by (Signature)	Relinquished by (Signature)	Date / Time	Received by (Signature)
Relinquished by (Signature)	Date / Time	Received by (Signature)	Relinquished by (Signature)	Date / Time	Received by (Signature)
Relinquished by (Signature)	Date / Time	Received for Laboratory by (Signature)	Date / Time	Remarks	

Distribution: Original Accompanies Shipment; Copy to Coordinator Field Files

Figure 2. Chain-of-Custody Record

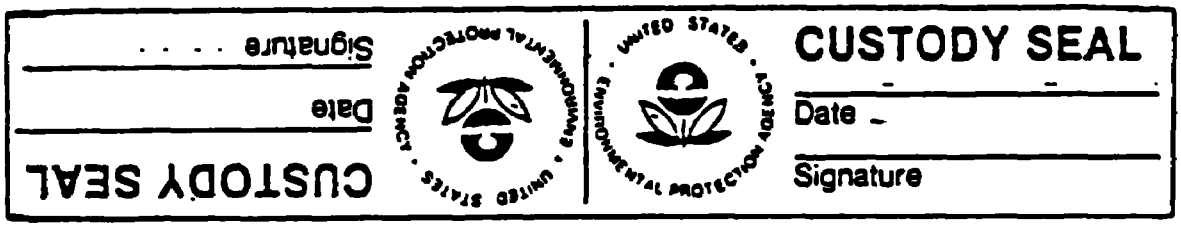


Figure 3. Example custody seal made of perforated paper stock.

1. Study plans or project plans.

2. Sample traffic records, weekly reports.

3. Custody records, sample tags, sample loop.

4. Laboratory logbooks, personal logbooks, instrument logbooks (or appropriate copies of logbook pages).

5. Laboratory data (sorted by sample), calibration and quality control data results.

6. Data summaries and reports.

7. All other documents, forms or records referencing the samples.

Figure 4. Example Document Inventory Format for each case.

APPENDIX C

ELUTRIATE AND FRACTIONATION METHODS

(PLUMB 1981)

Elutriate test

The elutriate test is a simplified simulation of the dredging and disposal process wherein predetermined amounts of dredging site water and sediment are mixed together to approximate a dredged material slurry.[2,20] The elutriate in the supernatant resulting from the vigorous 30-min shaking of one part sediment from the dredging site with four parts water (vol/vol) collected from the dredging site followed by a 1-hr settling time and appropriate centrifugation and 0.45 μ filtration. Thus, it will be necessary to collect both water and sediment samples to perform the elutriate test. When evaluating a dredging operation, the sediment should be collected at the dredging site and the water should be collected at the dredging and the disposal site. To evaluate a fill material activity, samples should be collected from the source of the fill material and the water should be collected from the disposal site.

Water sample collection. Collection should be made with an appropriate noncontaminating water sampling device. Either discrete samplers such as Kemmerer or Van Dorn samplers or continuous collectors such as submersible pumps may be used. The volume of water required will depend on the number of analyses to be performed. For each sample to be subjected to elutriate testing, it is suggested that a minimum of 4 ℓ be collected at the disposal site and 8 ℓ be collected at the dredging site to evaluate a dredging operation and/or 12 ℓ be collected at the disposal site to evaluate a fill material disposal operation. This will provide 4 ℓ of water for analyses and sufficient water to prepare triplicate 3-ℓ elutriates. (Each elutriate should yield 2.0 to 2.2 ℓ of standard elutriate for analysis.) If the samples are to be analyzed for trace organics or a large number of constituents, a proportionately larger initial sample should be collected.

Samples must be stored in glass containers if trace organic analyses are to be performed. Generally, either plastic or

glass containers may be used for other parameters. The samples should be maintained at 4°C until analyzed but never frozen. The storage period should be as short as possible to minimize changes in the characteristics of the water. Disposal site water should be analyzed or split and preserved immediately. The remainder of the water should be used in the elutriate test, which should be processed within 1 week of collection.

Sediment sample collection. Samples should be taken from the fill or the dredging site with a grab or a corer. Approximately 3 ℓ of sediment or fill material would provide sufficient sample to prepare triplicate 3-ℓ elutriates. Again, if the resultant standard elutriates are to be analyzed for trace organics or a large number of constituents, a proportionately larger initial sample should be collected.

Samples may be stored in plastic bags, jars, or glass containers. However, if trace organic analyses are to be performed, glass containers with teflon-lined lids are required. A special precaution that must be taken with sediment samples is to ensure that the containers are completely filled with sample and that air bubbles are not trapped in the container. This step is necessary to minimize sample oxidation that could influence elutriate test results.[2,22]

The samples should be stored immediately at 4°C. They must not be frozen or dried prior to use. The storage period should be as short as possible to minimize changes in the characteristics of the sediment. It is recommended that samples be processed within 1 week of collection.

Apparatus. The following apparatus are required to perform the elutriate test. Prior to use, all glassware, filtration equipment, and filters should be washed with 5 to 10 percent (or stronger) hydrochloric acid (HCl) and then rinsed thoroughly with deionized water. The necessary apparatus include:

a. Acid-rinsed plastic bottles for collection of water samples.

b. Plastic jars or bags ("Whirl-Pak," plastic freezer containers, etc.) for collecting dredged or fill material samples.

c. Laboratory shaker capable of shaking 2-ℓ flasks at approximately 100 excursions/minute. Box type or wrist-action shakers are acceptable.

d. Several 1-ℓ graduated cylinders.

e. Large (15 cm) powder funnels.

f. Several 2-ℓ, large-mouth graduated Erlenmeyer flasks.

g. Vacuum or pressure filtration equipment, including vacuum pump or compressed air source, and an appropriate filter holder capable of accomodating 47-, 105-, or 155-m-diameter filters.

h. Membrane filters with a 0.45-μ pore-size diameter. The filters should be soaked in 5 M HCl for at least 2 hr prior to use.

i. Centrifuge capable of handling six 1- or 0.5-ℓ centrifuge bottles at 3000 to 5000 rpm. International Model K or Sorval Super Speed are acceptable models.

j. Wide-mouth, 1-gal capacity glass jars with teflon-lined screw-top lids for use as sample containers when samples are to be analyzed for trace organics. (It may be necessary to purchase jars and teflon sheets separately; in this case, the teflon lid liners may be prepared by the laboratory personnel.)

Test procedure. The stepwise test procedure is given below:

a. Subsample a minimum volume of 1 ℓ each of dredging site and disposal site water. If it is known in advance that a large number of measurements are to be performed, the size of each subsample should be increased to meet the anticipated needs.

b. Filter an appropriate portion of the disposal site water through an acid-soaked 0.45-μ pore-size membrane filter that has been prerinsed with approximately 100 ml of disposal site water. The filtrate from the rinsing procedure should be discarded.

c. Analyze the filtered disposal site sample as soon as possible. If necessary, the samples may be stored at 4°C after splitting and the appropriate preservatives have been added (Table 2-4). Filtered water samples may also be frozen with no apparent destruction of sample integrity.

d. Repeat steps a, b, and c with dredging site water. This step is omitted with a fill material sample.

e. Subsample approximately 1 ℓ of sediment from the well-mixed original sample. Mix the sediments and unfiltered dredging site water in a volumetric sediment-to-water ratio of 1:4 at room temperature (22 ± 2°C). This is best done by the method of

volumetric displacement.[23] One hundred mililiters of unfiltered dredging site water is placed into a graduated Erlenmeyer flask. The sediment subsample is then carefully added via a powder funnel to obtain a total volume of 300 ml. (A 200-ml volume of sediment will now be in the flask.) The flask is then filled to the 1000-ml mark with unfiltered dredging site water, which produces a slurry with a final ratio of one volume sediment to four volumes water.

This method should provide 700 to 800 ml of water for analysis. If the analyses to be run require a larger volume of water, the initial volumes used to prepare the elutriate slurry may be proportionately increased as long as the solid-to-liquid ratio remains constant (e.g. mix 400 ml sediment and 1600 ml unfiltered dredging site water). Alternately, several 1-ℓ sediment/dredging site water slurries may be prepared as outlined above and the filtrates combined to provide sufficient water for analysis. The procedure continues as follows:

f. (1) Cap the flask tightly with a noncontaminating stopper and shake vigorously on an automatic shaker at about 100 excursions per minute for 30 min. A polyfilm-covered rubber stopper is acceptable for minimum contamination.

(2) During the mixing step given above, the oxygen demand of the dredged material may cause the dissolved oxygen concentration in the elutriate to be reduced to zero. This change can alter the release of chemical contaminants from dredged material to the disposal site water and reduce the reproducibility of the elutriate test.[21] If it is known that anoxic conditions (zero dissolved oxygen) will not occur at the disposal site or if reproducibility of the elutriate test is a potential problem, the mixing may be accomplished by using a compressed air-mixing* procedure instead of the mechanical mixing described in Step f (1). After preparation of the elutriate slurry, an air-diffuser tube is inserted almost to the bottom of the flask. Compressed air should be passed through a deionized water trap and then through the diffuser tube and the slurry. The flow rate should be adjusted to agitate the mixture

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* This procedure can cause the loss of highly volatile chemical constituents. If volatile materials are of concern, compressed air mixing should not be used.

vigorously for 30 min. In addition, the flasks should be stirred manually at 10-min intervals to ensure complete mixing.

g. After 30 min of shaking or mixing with air, allow the suspension to settle for 1 hr.

h. After settling, carefully decant the supernatant into appropriate centrifuge bottles and then centrifuge. The time and revolutions per minute during centrifugation should be selected to reduce the suspended solids concentration substantially and, therefore, shorten the final filtration process. After centrifugation, vacuum or pressure filter approximately 100 ml of sample through a 0.45-μ membrane filter and discard the filtrate. Filter the remainder of the sample to give a clear final solution (the standard elutriate) and store at 4°C in a clean, noncontaminating container in the dark. The filtration process is intended for use when the standard elutriate is to be analyzed for conventional chemical contaminants. When the elutriate is to be analyzed for organic contaminants and PCB's, filtration should not be used since organic concentrations can be reduced by sorption. Centrifugation should be used to remove particulate matter when the standard elutriate is to be analyzed for specific organics.

i. Analyze the standard elutriate as soon as possible. If necessary, the samples may be stored at 4°C after splitting and the appropriate preservatives have been added.

j. Prepare and analyze the elutriate in triplicate. The average of the three replicates should be reported as the concentration of the standard elutriate.

Sediment fractionation

Chemical constituents associated with sediments may be distributed in many chemical forms. The purpose of a fractionation procedure is to better define this distribution. This objective is achieved by leaching a sample with a series of successively harsher extraction agents. Reagents used in the procedure to be described below consist of interstitial water, ammonium acetate, hydroxylamine, hydrogen peroxide, citrate-dithionate, and hydrofluoric acid-nitric acid.

The premise of the fractionation procedure is that a specific geochemical phase is defined by a specific chemical

extraction agent. Thus, the ammonium acetate extract is referred to as the exchangeable phase, and the citrate-dithionate extract is referred to as the moderately reducible phase. These relationships have not been rigorously demonstrated and, therefore, the fractions are only operationally defined.

The use of fractionation results at this time appears to be limited to supporting other studies. That is, results have greater value in sediment research studies than in the regulatory decision-making process. Limited results with sediment fractionation data suggest a higher correlation with the more labile sediment phases (interstitial water, exchangeable phase) and elutriate test results[11] and long-term water quality changes.[10]

A major limitation of the fractionation procedure is that previous experience is limited to heavy metals and nutrients. A broad spectrum analysis of the individual fractions has been limited by small sample size, particularly the interstitial water fraction.

Sample collection. Samples should be collected with a grab or a corer. Because the distribution of sediment-associated chemicals can be altered by processes such as drying and oxidation, samples should be kept wet and exposure to the atmosphere should be minimized. Samples collected with a grab or dredge must be quickly transferred to a container and air bubbles must be excluded from the container. Corer samples should be sealed in the core liner and returned to the laboratory in an upright position. Previous studies have shown that a 15-cm section from a 7.5-cm-diameter core can provide sufficient material to perform fractionation studies for eight metals and four nutrients.[11]

The samples should be stored immediately at 4°C. They must not be frozen or dried prior to use. The storage period should be as short as possible to minimize changes in the distribution of chemical constituents in the sediments. It is recommended that samples be processed within 1 week of collection.

Apparatus. The following apparatus is required to perform the elemental partitioning procedure. Prior to use, all glassware, filtration equipment, and filters should be washed with 5 to 10 percent HCl and then rinsed thoroughly with deionized water. This list of

equipment includes:

a. Plastic jars or bags ("Whirl-Pak," plastic freezer containers, etc.) for collecting grab or dredge samples or polyethylene liners for the collection of core samples.

b. Glove box or disposable glove bag.

c. Polarographic oxygen analyzer or alternate method to confirm the absence of oxygen in glove bag.

d. Utensils for splitting cores and handling samples in the glove bag.

e. 250-ml and 500-ml polycarbonate centrifuge bottles.

f. Refrigerated centrifuge.

g. Vacuum filtration apparatus.

h. 150-ml and 120-ml polyethylene storage bottles.

i. Blender or porcelain mortar and pestle.

j. Top-loading balance.

k. Weighing dishes.

l. Digestion block or hot plate.

m. Teflon beakers.

n. 50-ml volumetric flasks.

Test procedure. A stepwise sediment fractionation procedure is given in the following paragraphs.

To begin the procedure, prepare a glove box or disposable glove bag. Flush the system with nitrogen gas and maintain a positive pressure nitrogen atmosphere. Oxygen-free conditions in the glove bag or box should be verified with a polarographic oxygen analyzer prior to sample processing. Initial sample handling and all steps in the interstitial water and ammonium acetate extractions should be conducted under a nitrogen atmosphere.

Acid wash all hardware to be used in the extractions in 6 N HCL and thoroughly rinse with distilled water to minimize sample contamination during processing.

The initial separation removes the interstitial water phase. This is accomplished by first placing the sealed sediment sample in the glove bag. After reestablishing the nitrogen atmosphere, extrude the sediment core from its liner into a flat plastic container. If the core is to be sectioned vertically, 15-cm sections of a 7.5-cm-diameter

core have been shown to provide sufficient material for the sequential fractionation procedure. Each core section should be split into halves with one half (approximately 300 cc) being used for the interstitial water testing and the remaining half used for all other analyses. Place the half section for the interstitial water analysis in an oxygen-free, polycarbonate 500-ml centrifuge bottle in the glove bag and seal. Centrifuge the sample in a refrigerated centrifuge (4°C) at 900 revolutions per minute (∿13,000 × g) for 5 min. This should be sufficient to recover 40 percent of the total sediment water. After centrifugation, return the sample to the glove bag and vacuum filter the interstitial water through a 0.45-μ pore-size membrane filter. Transfer the filtered sample to an acid-washed polyethylene bottle and acidify to pH 1 with HCl for preservation.

If the sediment sample is not to be sectioned vertically, decant excess water and blend the core or dredge sample. Place approximately 300 cc of the blended sample in an oxygen-free, polycarbonate 500-ml centrifuge bottle and seal. Centrifuge this sample for 5 min at 900 revolutions per minute (13,000 × g) in a refrigerated centrifuge (4°C) and then filter through a 0.45-μ pore-size membrane filter under a nitrogen atmosphere. The filtered sample may be analyzed immediately or split for preservation and storage.

The exchangeable phase is determined on the unused half of the wet sediment sample that was blended for interstitial water analysis. Blend the wet sediment with an electrically driven polyethylene stirrer contained in the glove bag. Remove a subsample of the homogenized sediment sample (blended core section, core, or grab sample) for percent solids determination.

Weigh a second subsample (approximately 20 g dry weight of each homogenized sediment section into an oxygen-free, tarred, 250-ml centrifuge tube containing 100 ml deoxygenated 1 N ammonium acetate, producing a suspension with an approximate solid-to-liquid ratio of 1:5. Adjust the pH of the acetate solution to the pH of the surface sediments. Seal the samples and then place on a wrist-action shaker for 1 hr. Centrifuge the samples at 6000 revolutions per minute for 5 min and return them to the glove bag for further processing under

the nitrogen atmosphere. Filter the sample through 0.45-μ pore-size membrane filters, retaining both the filtrate and the solid residue.

The filtrate may be analyzed immediately or split and preserved for specific constituents, as discussed for water samples. This extract will also include the interstitial water components since a fresh blended sediment sample was used. Therefore, measured concentrations should be reduced to compensate for the interstitial water. This can be accomplished as:

$$\text{Wt exchangeable material} = (\text{Vol ext})\,(\text{Conc ext}) - \frac{(\text{Wt Sample})\,(1-\%\ \text{Solids})}{(\text{density water})}\,(\text{IWC}) \tag{3}$$

where

(Vol ext) = volume of ammonium acetate extract

(Conc ext) = analytical concentration in ammonium acetate extract

(Wt Sample) = wet weight of sample for ammonium acetate extraction

% Solids = percent solids in sample

(density water) = density of water at temperature of sample

(IWC) = interstitial water concentration of sample

The <u>easily reducible phase</u> is performed with the solid residue from the exchangeable phase determination. This step and all subsequent steps in the fractionation procedure can be conducted outside the glove bag. Add 50 ml N_2 sparged distilled-deionized water to the centrifuge tube containing the solid residue from the 1 <u>N</u> ammonium acetate extraction. Agitate the sample with a stainless steel spatula or a glass-stirring rod to ensure good washing efficiency. Centrifuge the suspension of 6000 revolutions per minute and discard the liquid phase. A portion of the solid residue will have to be set aside at this point for a redetermination of percent solids.

Blend the remaining sediment residue and transfer a 2-g (dry weight equivalent) subsample to a 250-ml Erlenmeyer flask. Add 100 ml of 0.1 <u>M</u> hydroxylamine hydrochloride-0.01 <u>M</u> nitric acid solution. The resultant suspension will have a solid-to-extractant ratio of approximately 1:50. Seal the sample and place the suspension on a wrist-action shaker (or equivalent) for 30 min. Centrifuge the sample

at 6000 revolutions per minute for 5 min. Decant and filter the liquid phase through 0.45-μ pore-size membrane filters. The filtrate may be treated as a water sample and analyzed immediately or split and preserved for specific constituents.

The solid residue is used in the organic and sulfide phase extraction. Wash the residue from the easily reducible phase with 50 ml distilled water. After agitating the suspension, centrifuge the sample at 6000 revolutions per minute for 5 min and discard the supernate. Subsample the residue for a percent solids determination so the organic and sulfide results can be expressed on a dry weight basis. Add 50 ml of 30 percent hydrogen peroxide to the washed residue and adjust the pH to 2.5 with HCl. (The purpose of the pH adjustment is to prevent any released metals from precipitating.) Digest the sample at 95°C for 6 to 8 hr. Add 100 ml of 1 N ammonium acetate buffered at pH 2.5 to the digestate and shake for 1 hr. Centrifuge the sample at 6000 revolutions per minute for 5 min and filter the sample through 0.45-μ pore-size membrane filters. Treat the filtrate as a water sample and analyze immediately or split and preserve as required. Retain the solid residue.

The next fraction in the sequence is the moderately reducible phase. Wash the organic and sulfide phase solid residue with 50 ml of distilled water; centrifuge as described earlier; and discard the supernate. Redetermine percent solids on a subsample of the residue. Add 100 ml of a citrate-dithionate solution (16 g sodium citrate + 1.67 g sodium dithionate/100 ml distilled water) and mechanically shake the suspension for 17 hr. Centrifuge the sample at 6000 revolutions per minute for 5 min and filter the supernate through a 0.45-μ pore-size membrane filter. Analyze the filtered sample for moderately reducible constituents and retain the solid residue for further treatment.

The sample for residual phase digestion is obtained by washing the moderately reducible phase residue with 50 ml of distilled water; centrifuging at 6000 revolutions per minute for 5 min; and discarding the supernate. Dry the residue at 105°C and transfer

a 0.5-g dry weight subsample to a teflon beaker. Add 15 ml hydrofluoric acid and 10 ml concentrated nitric acid; cover the beaker; and digest at 175°C. After evaporation to near dryness, add 8 ml fuming nitric acid stepwise in 2-ml increments. Continue evaporation to near dryness. Add 6 N HCl to dissolve the residue, heating if necessary. Quantitatively transfer the solution to a 50-ml volumetric flask and dilute to volume. Analyze the sample immediately or preserve subsamples for specific constituents.

A schematic flow diagram for the fractionation procedure is presented in Figure 2-2. When this procedure is used, a built-in quality control check is to total each of the operationally defined phases and compare to a total digest of the sample. The data should be considered suspect if they differ by more than 5 to 10 percent.

Bulk analysis

A bulk analysis provides a measure of the total concentration of a specific constituent in the sample being analyzed. This is accomplished by subjecting a sample to strong oxidation, acid digestion, or organic solvent extraction. The procedure is similar to that used for the residual phase digestion in the elemental partitioning procedure discussed earlier. Total sediment concentrations can be used to compare different sites and to identify major point sources. However, because of the harshness of the extraction procedure, information on chemical distribution and/or potential environmental impact is lost.

Sample collection. Samples for total analyses may be collected with a dredge or grab sampler or a core sampler. Approximately 1 to 2 ℓ of sediment or fill material should be taken from the proposed project site and placed in plastic jars or containers. If trace organic constituents are to be determined, the sample should be stored in a glass container or a second sample of approximately the same size should be collected and stored separately in glass containers. Samples should be stored at 4°C.

Upon reaching the laboratory, sediment samples may be stored wet, air dried, or frozen. The selection between these preservation techniques should be based primarily on the specific

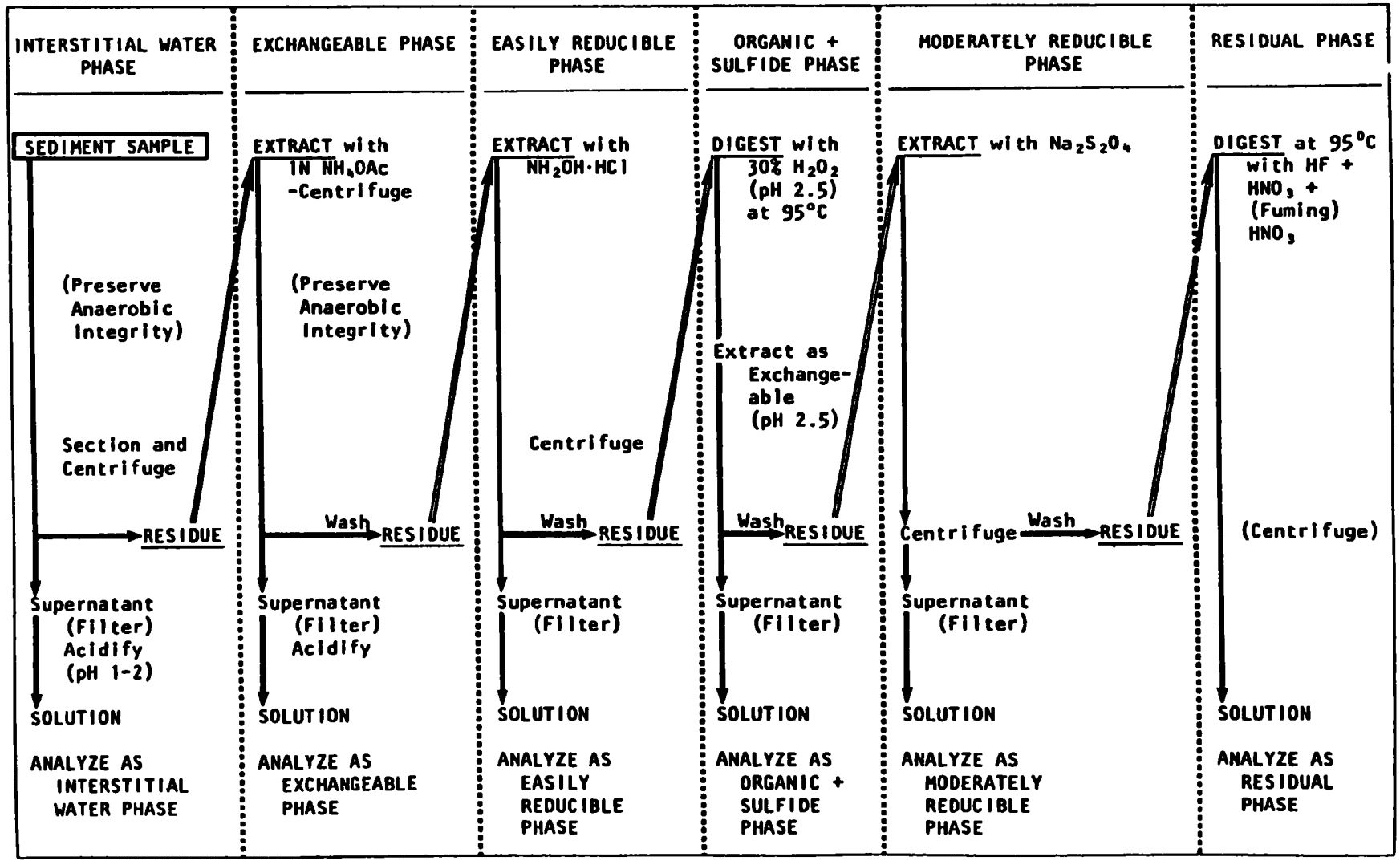

Figure 2-2. Elemental partitioning for sediment characterization

parameter to be determined and, secondarily, on personal choice. Several parameters such as pH, redox, total solids, and volatile solids must be run on wet samples. Other parameters may change due to oxidation (chlorine demand, BOD, COD, SOD, sulfides), volatilization (phenolics, volatile solids), or chemical instability (carbamates, herbicides). Samples to be analyzed for these parameters should be processed as soon as possible using subsamples of the original wet sample. Samples to be analyzed for particle size (dispersed), total organic carbon (TOC), metals (except possibly mercury), chlorinated hydrocarbon pesticides, and PCB's may be stored wet, dried, or frozen.

Apparatus. The specific equipment necessary will vary depending on the chemical constituent(s) to be analyzed in the total sediment digest or the total sediment organic extract. Specific needs and cleanup procedures are discussed with each parameter in Section 3.

Test procedure. The following stepwise procedure is recommended for the processing of sediment or fill material samples to be analyzed for total or bulk content:

a. Decant any overlying water that may have been collected with the dredge or corer.

b. Blend the dredge, core, or sectioned core sample.

c. Transfer an aliquot of the homogenized sample to a tarred weighing dish and weigh. Dry the sample at 105°C to a constant weight. This information will allow calculation of percent solids in the sample and to report subsequent bulk analysis results on a milligram-per-kilogram dry weight basis. The dried sample from the percent solids determination may be subjected to further chemical analysis for those parameters not affected by the drying process.

   If volatile solids are to be determined, record the weight of the crucible and the dried sample used in the percent solids determination. Place the sample in an electric muffle furnace and ignite the sample at 600°C for 60 min. Remove the sample from the furnace, allow to cool, and desiccate for 30 min prior to weighing. Report the weight lost on ignition as percent volatile solids.

d. Transfer a second subsample of the blended sample to a suitable container for pH and oxidation-reduction (redox) potential determinations. The sample size

should be sufficient to allow the electrodes to be inserted to a depth of 4 to 6 cm. Allow sufficient time for the electrode responses to stabilize and record the respective pH and redox values.

e. Set aside subsamples of the wet, blended sample for the analysis of time-dependent or unstable chemical constituents. Parameters in this category include biological oxygen demand, chemical oxygen demand, sediment oxygen demand, chlorine demand, herbicides and carbamates, phenolics, sulfides, nitrogen, phosphorus, and oil and grease. Thus, as many as 12 subsamples (if all listed analyses are to be performed) will be required. Suggested sample sizes for each aliquot are presented in Figure 2-3. These analyses should be initiated as soon as possible to minimize the effects of sample alteration due to handling and storage.

f. Set aside separate subsamples for the analysis of particle size, carbon, metals, and chlorinated hydrocarbons. However, because of the increased stability of these constituents (relative to those in Step e, above), the aliquots may be taken from the initial wet, blended sample, or a sample that has been dried or frozen for storage. Required subsample sizes are presented in Figure 2-3.

Individuals performing bulk analysis of sediment samples should be aware of the fact that analytical results may be affected by sample handling and storage procedures. The following special caveats are maintained here because of the importance of this fact and again with the appropriate analytical procedure in Section 3:

a. It is preferable to determine Eh and pH values in the field as soon as the sample is collected since there is no way to stablize these parameters. If this is not possible, these parameters should be determined as soon as possible in the laboratory using a wet sample. Sample handling should be kept to a minimum to avoid sample dehydration or sample oxidation.

b. Percent solids and specific gravity also must be determined on a sample of original moisture content. The sample should be handled in such a manner to minimize water loss and sample dehydration.

c. Cation exchange capacity can be influenced by sample drying. Therefore, it is recommended that this parameter be determined on original moisture content samples.

d. Chlorine demand, biological oxygen demand, chemical oxygen demand, and sediment oxygen demand are all

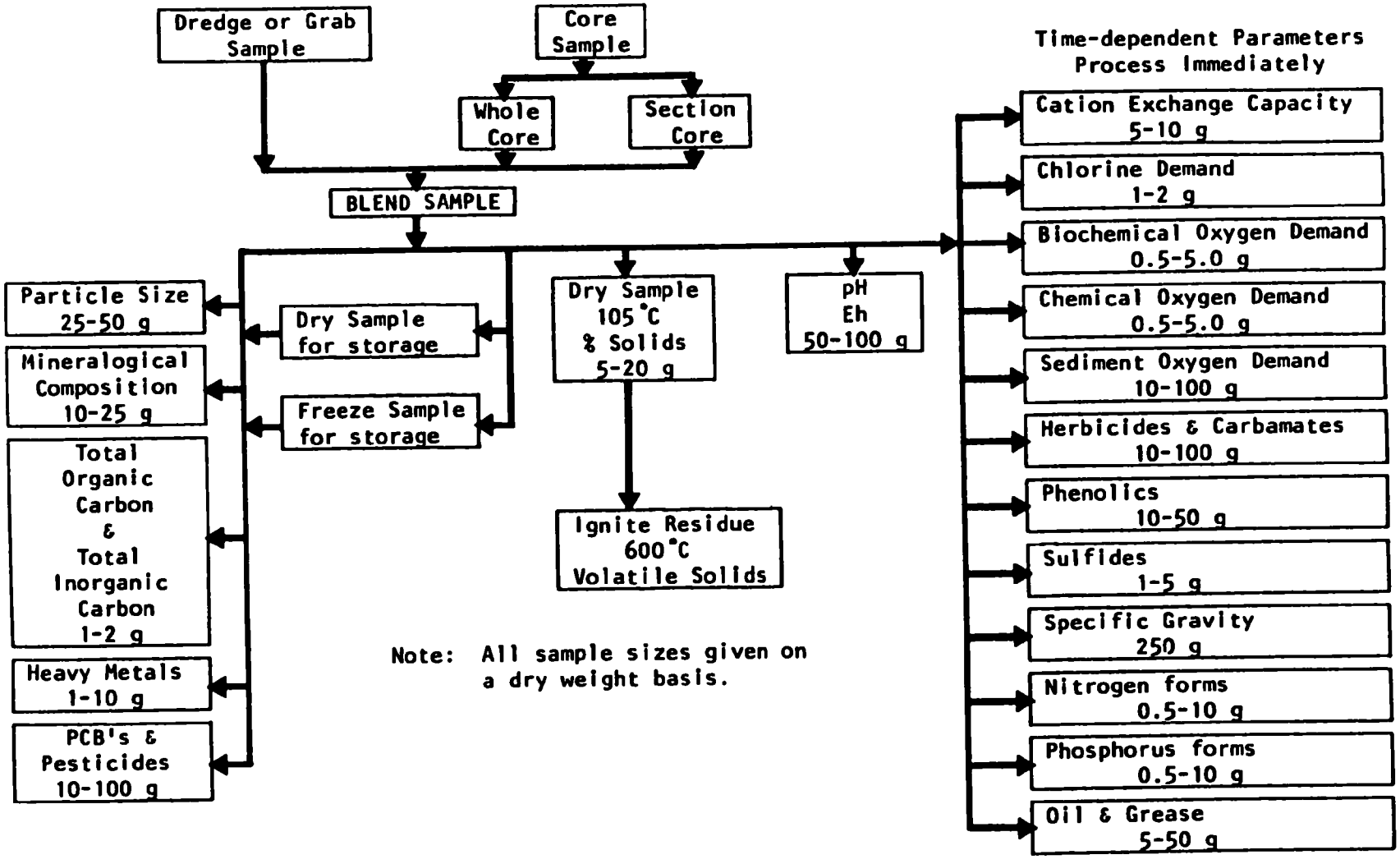

Figure 2-3. Sediment sample splitting for bulk analysis

measures of the reducing capacity of the sample being analyzed. Since sediments are frequently reduced and contain elevated concentrations of ferrous iron, manganous manganese, and sulfide that can be oxidized by atmospheric oxygen, these parameters should be run on wet samples.

e. Herbicides and carbamates are chemically unstable with relatively short half-lives. Immediate extraction of the original sample with methylene chloride reduces the possibility of chemically or biologically catalyzed decomposition and increases herbicide and carbamate stability.

f. Phenolic compounds may be lost by volatilization during storage. Therefore, samples to be analyzed for phenols should not be dried and storage time should be minimized. If immediate analysis is not possible, storage by freezing may be acceptable. Subsequent sample thawing should be accomplished at low temperature to reduce phenol loss by volatilization.

g. Sulfides in the sample may be lost by volatilization and oxidation. It is recommended that sample contact with atmospheric oxygen be minimized between sample collection and analysis to reduce this effect. This can best be accomplished by excluding air bubbles from sample containers and minimizing sample storage time.

h. Some forms of nitrogen that are expected to occur in sediments (nitrites) are unstable in the presence of oxygen and can be lost on sample drying. In addition, sample composition may be altered by the uptake or loss of volatile ammonia. Therefore, sample processing should begin as soon as possible using a sample of original moisture content.

i. The distribution of phosphorus forms may be altered by changes in other sample constituents. For example, the oxidation of iron in a sample may precipitate soluble phosphate. Therefore, if soluble phosphate is to be determined, wet samples should be processed as soon as possible. If total phosphate is the only parameter of concern, analysis can be conducted on a wet, dried, or frozen sample.

j. The oil and grease content of samples may be reduced due to volatilization. Consequently, sample drying prior to analysis is not recommended.

k. The selection of a storage technique for samples to be analyzed for particle size depends on the method of analysis. If apparent particle size is to be run, a wet sample should be used. However, if dispersed particle size is to be run, a wet, dried, or frozen sample may be used.

l. Most of the heavy metals are stable and samples scheduled for analysis can be stored in a wet, dried, or frozen state. The selection of a storage method can affect the distribution of a metal among various forms, but the total concentration should be unaffected. Two possible exceptions are mercury and selenium, which can be lost by volatilization. This particularly is true if the samples are dried above 60°C.

m. Chlorinated hydrocarbon pesticides and PCB's are stable and probably unaffected by the method of sample storage. Improved stability can be achieved by immediate extraction of the original sample with an organic solvent and is suggested, but not essential.

n. The analysis of a sediment or fill material sample for specific constituents will require a sample digestion or sample extraction technique. Since the selection of a digestion solution or solvent is dependent on the analysis to be performed, this information is presented with the specific analytical techniques.

Summary

Dredged material may be subjected to several types of testing. This section has provided guidance for conducting elutriate testing, elemental partitioning, and bulk analysis of sedimentary samples. Since each of these procedures measures a different property of the sample, different storage requirements are required for samples to be subjected to each testing procedure. Therefore, this section has also provided detailed guidance for the handling of sedimentary samples from the time of collection until the time of analysis.

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APPENDIX D

TOTAL ACID DIGESTION METHOD FOR SEDIMENT

(RANTALA AND LORING 1975)

REAGENTS

1. Concentrated hydrofluoric, hydrochloric, and nitric acids [ultrex grade (Baker) or equivalent]

2. 2.5 percent boric acid - dissolve 50 g [ultrex grade (Baker) or equivalent] boric acid in 2 L distilled-deionized water.

3. 30 percent hydrogen peroxide [ultrex grade (Baker) or equivalent].

PROCEDURE

1. If mercury is to be analyzed, a separate, 0.4-g (wet) aliquot is digested and analyzed by the cold vapor AA technique detailed in Appendix B. (Note that the sample weight must be doubled to achieve the 0.01-mg/kg dry weight detection limit specified in Table 6 of Appendix A.)

2. Dry a representative portion of sediment overnight at 60°C. Grind to 100 mesh.

3. Weigh 200-mg dried sample into a decomposition vessel. (Vessels of various sizes may be obtained from: (a) Savillex Corp., 5325 Hwy. 101, Minnetonka, MN, USA 55343; (b) H.K. Morrison and Sons Ltd., Mount Uniacke, N.S., Canada; (c) Parr Instrument Company, 211-53rd St., Moline, IL, USA 61265; (d) Uniseal Decomposition Vessels Ltd., P.O. Box 9463, 31 094 Haifa, Israel - distributed in the USA by Columbia Organic Chemicals Co., P.O. Box 1045, Camden, SC 29020.) Add 0.5 mL conc. HNO3.

4. In a fume hood, warm on a hot plate at low setting and open to the atmosphere until the brown NO_2 fumes are absent. To aid digestion, a few drops of 30 percent H_2O_2 may be added.

5. Cool to room temperature. Add 0.75 mL conc. HCl and 3.0 mL conc. HF. Seal the digestion vessel.

6. Heat in an oven for 2 h at the digestion temperature recommended by the manufacturer ($105^{o}C$ - $130^{o}C$). (Note: a longer digestion time may be necessary for some sediments if visible particulates are present <u>after</u> the addition of boric acid. Some fluorides, such as CaF_2 are insoluble in the digestion acids and appear as a greyish sludge. These will dissolve upon addition of boric acid. Also, flakes of black, undigested carbonaceous matter may also be present. These will not dissolve upon boric acid addition, but will not interfere with the analysis.)

7. Remove vessel from oven and cool to room temperature. Add 20 mL of 2.5 percent boric acid. Reseal vessel and return to oven at recommended temperature for 1 h.

8. Remove vessel from oven and cool to room temperature. Quantitatively transfer contents to a 25 mL polyethylene volumetric flask and bring to the mark with distilled-deionized water.

9. Digestate may be analyzed directly using the recommended atomic spectroscopic instrumental techniques.

MULTI-ELEMENT ANALYSIS OF SILICATE ROCKS AND MARINE SEDIMENTS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

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ABSTRACT

A rapid method is described for the determination of 21 elements in silicate rocks and marine sediments by flame atomic absorption. The method offers good accuracy and precision in major, minor, and trace element determinations and is very suitable for marine geochemical studies. Precision and accuracy have been evaluated by analyzing seven U.S.G.S. rock standards.

RESUME

On décrit une méthode rapide pour la détermination de 21 éléments dans des roches silicates et des sédiments marins, par absorption atomique de flamme.

La méthode donne une bonne exactitude et une bonne précision pour la détermination d'éléments majeurs, mineurs et traces et est très adaptée à des études de géochimie marine. L'évaluation de la précision et de l'exactitude a été faite par l'analyse de sept standards de roche USGS.

ZUSAMMENFASSUNG

Es wird eine rasche Methode zur Bestimmung von 21 Elementen in Silikatgesteinen und Meeres-Sedimenten mittels Flammen-Atomabsorption beschrieben Die Methode bietet für die Bestimmung der Haupt.-Neben.-und Spurenelemente eine gute Richtigkeit und Präzision und eignet sich ausgezeichnet für geochemische Studien im Bereich des Meeres Präzision und Richtigkeit wurden durch Analyse von 7 USGS Gesteins-Std. bestimmt.

INTRODUCTION

Recent trends in the field of atomic absorption spectroscopy have been toward flameless atomization. Severe matrix problems are encountered, however, in samples such as silicate rocks and marine sediments. Remedies such as selective volatilization and the method of standard additions have failed to eliminate these problems in complex solutions (1).

Flame atomization, for which matrix interferences can generally be controlled satisfactorily, is one of the most suitable methods for the analysis of silicate rocks and marine sediments.

The method described here consists of an acid decomposition in a Teflon bomb followed by flame atomic absorption for the determination of 21 elements in silicate rocks and marine sediments. It is more comprehensive in terms of number of elements than other reported schemes based on HF decomposition (2,3,4).

For the assessment of relative accuracy and the precision of this method, U.S.G.S. rock standards granite G-2 and diabase W-1 have been analyzed for 21 major, minor, and trace elements: Al, Ca, Fe, K, Mg, Mn, Na, Si, Ti, Ba, Be, Co, Cr, Cu, Li, Ni, Pb, Rb, Sr, V and Zn.

In addition, U.S.G.S. rock standards andesite AGV-1, basalt BCR-1, dunite DTS-1, granodiorite GSP-1 and peridotite PCC-1 were analyzed for 12 trace elements.

For some elements, notably Ba, Be, Cr, Pb, Rb and V, which were determined by this method, no atomic absorption values were reported by Flanagan in his compilation of the rock standard data (5).

PROCEDURE

Sample Preparation

Table 1 shows the analytical scheme for the major and trace elements.

For the major and some minor elements, a 0.1-g sample is decomposed with HF and aqua regia in a Teflon vessel* (6) and initially diluted to 100 ml. From this solution Mn, Si, and also Ni for DTS-1 and PCC-1 are determined directly.

Solid KCl is added to separate aliquots to give a potassium concentration of 1500 ppm, and these are analyzed for Ba, Sr, and Ti.

For the determinations of Al, Ca, Fe, Mg, and Na, a further 20X dilution is made to give the desired dilution factor of $2X10^4$ for these elements in the KCl (1500 ppm) matrix. A similar dilution is also made for the determination of K by substituting NaCl as the diluent.

For trace element determinations, a separate 1-g sample

TABLE I
Sample Preparation Diagram for Silicate Rock Analyses

0.1-g sample → Teflon vessel decomposition	→1000X dilution	→ Mn, Si
	→1000X dilution in 1500 ppm K	→ Ba, Sr, Ti
	→2000X dilution in 1500 ppm K	→ Al, Ca, Fe, Mg, Na
	→2000X dilution in 1500 ppm Na	→ K
1-g sample → Teflon vessel decomposition → 100X dilution		→ Be, Co, Cr, Cu, Li, Ni, Pb, Rb, V, Zn

*Available from H. K. Morrison and Sons Ltd., Mount Uniacke, Hants Co., N. S., Canada.

is decomposed in the Teflon vessel and diluted to 100 ml. From this solution Be, Co, Cr, Cu, Li, Ni, Pb, Rb, V, and Zn are determined directly.

Decomposition Procedure

A finely ground sample of 0.1 g or 1 g is weighed accurately in the Teflon vessel and wetted 1 ml of aqua regia. Six ml of concentrated HF are added slowly, and the cover is placed on tightly. The vessel is immersed in a water bath (90°C-100°C) for 1 hr. Care should be taken that the vessels remain immersed during the entire heating period. Following the heating, the vessels should immediately be removed from the water bath and placed in cold water. The water level should not reach the threaded cover. These precautions are necessary to avoid sucking water into the vessels by the vacuum created during cooling. After cooling, the contents are washed into a 100-ml polypropylene volumetric flask containing 5.6 g H_3BO_3 that has been shaken with approximately 20 ml of H_2O. The flask is shaken to complete the dissolution and made up to volume. The solutions are stored in polypropylene bottles.

Sample solutions thus prepared are extremely stable. In more concentrated trace element solutions, a gelatinous precipitate of borosilicates will form due to the high concentration of silicon. This has no adverse effect on trace element determinations (6), and the removal of the bulk of silicates is desirable.

Standard Preparation

Single-element standards for major and minor elements can be used. For the sake of convenience, however, a combined stock standard solution was prepared. This, as well as other standards, was prepared from 1000-ppm standards. The 1000-ppm standards were prepared either in the laboratory or obtained commercially. No specific details are given here for preparing these solutions. Such information is readily available (7).

The exceptions are the Si, Ti, and Cr standards which are described below. A combined stock standard solution of 300 µg/ml Al and 100 µg/ml Ca, Fe, K, Mg, Mn, and Na was prepared. This stock standard was diluted 25, 50, 75, 100, 200, and 400 times for working standards. All working standards were prepared in 1500 ppm K except standards for K which were prepared in 1500 ppm Na. The decomposition blank contained 1 ml aqua regia, 6 ml concentrated HF, and 5.6 g H_3BO_3/100 ml.

A 1000-ppm Si standard was prepared from a silica rod by the same decomposition procedure as described for the samples. Working standards of 200-400 ppm Si were prepared in a decomposition blank solution.

A 1000-ppm Si standard was prepared from a silica rod by the same decomposition procedure as described for the samples. Working standards of 200-400 ppm Si were prepared in a decomposition blank solution.

The 1000-ppm Ti standard was prepared from TiO_2 by the same decomposition procedure described for the samples. Working standards of 2-10 ppm Ti were prepared in the decomposition blank with 1500 ppm K.

Single-element standards were prepared for Ba, Be, Cr, Rb, Sr, and V. A combined stock standard solution of 50 µg/ml each was prepared for Co, Cu, Li, Ni, Pb, and Zn. Working standards of 0.1-2.0 ppm for these elements were prepared in the decomposition blank and are stable for several months.

Working standards of 0.1-2.0 ppm Ba were prepared in the decomposition blank with 1500 ppm K.

In preparing Be standards it is advisable to use commercially available stock standard solutions since beryllium dust is potentially toxic. Working standards of 5-30 ppb Be were prepared in the decomposition blank.

A 1000-ppm Cr standard was prepared from $K_2Cr_2O_7$. Working standards of 0.1-1.5 ppm Cr were prepared in the decomposition blank. The best sensitivity for Cr is obtained in the air-acetylene flame, but it is first necessary to reduce Cr^{6+} to Cr^{3+} in the solution because the standards give a low absorption if the Cr is not reduced. This reduction was carried out by adding 2 ml perchloric acid and 1 ml 30% H_2O_2 to 20 ml of 1000 ppm Cr^{6+}, followed by heating and dilution to 1000 ml to obtain a 20-ppm Cr^{3+} standard (8). This standard was diluted with the decomposition blank solution for working standards. Reduction of Cr^{6+} to Cr^{3+} is not necessary for Cr determination in a nitrous oxide-acetylene flame, but this method is less sensitive.

Working standards of 0.2-3.0 ppm Rb were prepared in the decomposition blank with sufficient K added to match the sample concentration. The absorption of Rb is suppressed by the decomposition blank solution and enhanced by K. Enhancement by K is progressive to about 600 ppm K, above which suppression by K occurs. Thus, the addition of excessive amounts of K results in a vastly suppressed absorption signal and cannot be recommended. Enhancement by Na is small. When 200 ppm of Na and K were added to a 1-ppm Rb standard in the decomposition blank, the combined enhancement was about 2% more than in 200 ppm K only. Since the determination is highly dependent on K concentration, it was first necessary to determine the K content of the samples and match it to the standards. Since the absorption of Rb was observed to be linear at least to 3 ppm Rb for a particular K concentration, several standard curves could be drawn by using just one standard in each case. Where large numbers of samples are analyzed, it is not practical to match the sample concentration of K in each standard, but the Rb concentration can be interpolated from other standard curves. It remains to be investigated whether another alkali metal could be used in large amounts to compensate for interferences without causing suppression of Rb absorption.

Working standards of 0.1-1.0 ppm Sr were prepared in the decomposition blank with 1500 ppm K.

Working standards of 0.3-5.0 ppm V were prepared in the decomposition blank with 100 ppm Al and 500 ppm K. Both Al and K enhance the vanadium absorption. No further enhancement was observed above 100 ppm Al and 500 ppm K.

APPARATUS

A Perkin-Elmer Model 303 atomic absorption spectrophotometer equipped with a Deuterium Background Corrector and a 10-mV full-scale deflection recorder was used for the determination of all elements except Pb which was determined using a Perkin-Elmer Model 306 atomic absorption spectrophotometer. Scale expansion was 10X or less for all determinations. Light sources for K, Na, and Rb were Osram arc-discharge lamps. A Perkin-Elmer EDL was the light source for Pb. The Be light source was a Westinghouse hollow cathode lamp, and the remaining elements were determined with Perkin-Elmer Intensitron® single-element hollow cathode lamps. Acetylene was the fuel for all elements. Air was used as the oxidant, except for Al, Ca, Mg, Si, Ti, Ba, Be, Sr, and V for which nitrous oxide was employed.

INTERFERENCES

Matrix interferences on major and minor elements are reduced by dilution to bring the concentration to the linear portion of the absorbance curve. Ionization is controlled by additions of 1500 ppm K or Na to standards and samples. Chemical suppression by elements such as silicon and aluminum on calcium and magnesium absorption was avoided by the use of a nitrous oxide-acetylene flame. This eliminated the need for lanthanum which is essential in an air-acetylene flame. No interferences were observed for Mn and Si in a fluoboric-boric acid matrix.

Ionization in Ba and Sr determinations was controlled by adding 1500 ppm K to samples and standards. Standards for Cr, Rb, and V require special attention as discussed under standard preparation. Molecular absorption is corrected by the Deuterium Background Corrector in Co, Ni, and Pb determinations. No interferences were observed for Be, Cu, Li, and Zn in a fluoboric-boric acid matrix.

RESULTS AND DISCUSSION

Table II indicates the relative accuracy of the major and minor element data for W-1 and G-2 by the comparison of our values to those compiled by Flanagan (9). The precision is expressed as a coefficient of variation. To obtain these data, 6 separate portions of W-1 and G-2 were analyzed.

Table III indicates the relative accuracy of the trace element determinations for seven U.S.G.S. standard rocks by comparison of our values to those compiled by Flanagan. The precision is expressed as a coefficient of variation (Table IV). These data are based on 3 separate portions of the rock sample

TABLE II
Major Elements in U.S.G.S. Standard Rocks (%)

		A	B	Coeff. Var. (%)
Al	W-1	7.94	7.68	1.5
	G-2	8.15	8.11	2.7
Ca	W-1	7.83	7.83	0.7
	G-2	1.39	1.40	1.9
Fe	W-1	7.76	7.96	0.4
	G-2	1.85	1.90	5.0
K	W-1	0.53	0.56	4.7
	G-2	3.74	3.62	1.2
Mg	W-1	3.99	3.86	0.1
	G-2	0.46	0.44	4.1
Mn	W-1	0.132	0.127	1.1
	G-2	0.026	0.025	4.4
Na	W-1	1.59	1.60	1.2
	G-2	3.02	2.94	0.8
Si	W-1	24.6	24.6	2.6
	G-2	32.3	31.5	0.8
Ti	W-1	0.64	0.67	1.5
	G-2	0.30	0.31	2.6

A Flanagan (8)
B This work

Close agreement with Flanagan's values can be noted for most elements. Zinc values for DTS-1 and PCC-1 are low. This could be due to incomplete decomposition of chromites. An appreciable amount of chromite which could not be decomposed entirely is contained in DTS-1 and PCC-1. therefore, it was not possible to determine Cr accurately in these samples.

Nickel values for AGV-1 and W-1 are comparatively low. An attempt was made to determine Ni without background correction. The positive error that resulted, however, was considerably larger than the negative error with background correction. It has been shown that very large positive or negative errors are possible in the determination of Pb, Zn, Ni, and Co in geological materials which depend upon the concentration of the trace elements and major elements in the sample solution (10). The dilution factor of 100X and the fluoboric-boric acid matrix used in this method minimize these errors.

The precision for major and minor elements is $\leq 5\%$ and for trace elements generally $<10\%$.

For most major elements the relative accuracy is comparable and precision improved over that reported by Buckley and Cranston. However, for trace elements present in low concentrations, both the relative accuracy and precision are significantly improved over the values reported by the above authors. No complete comparison can be made at this time with other schemes due to the lack of published statistical data on all the elements we have studied by this method.

The method described here is ideal for major geochemical studies on marine sediments (11) and is now used in this laboratory for routine analyses. Although the organic matter is not completely oxidized during the decomposition, the residue is generally $<3\%$, and its elemental contribution is neligible. The method is rapid as a large number of inexpensive Teflon vessels can be used simultaneously and the dilutions carried out with commercial sampler-diluters.

CONCLUSION

The method described for multi-element analyses of silicate rocks and marine sediments by flame atomic ab-

TABLE IV
Precision (Coefficient of Variation) for the Determination of Trace Elements in U.S.G.S. Standard Rocks (%)

	AGV-1	BCR-1	W-1	DTS-1	G-2	GSP-1	PCC-1
Ba	2.0	5.7	3.2	—	1.1	1.4	—
Be	2.8	2.2	4.0	—	1.7	1.9	—
Co	14	7.3	2.4	0.9	7.9	12	0.9
Cr	5.6	3.9	0.5	—	6.1	1.9	—
Cu	4.2	6.7	3.7	4.2	4.3	4.9	5.6
Li	2.6	2.5	2.2	6.4	0.8	1.5	10
Ni	—	—	4.5	—	—	—	—
Pb	4.3	15	—	—	11	8.6	—
Rb	2.3	1.2	4.1	—	2.1	0.5	—
Sr	2.2	2.9	5.1	—	1.6	3.5	—
V	1.2	2.4	0.7	—	6.0	4.3	7.5
Zn	1.3	1.6	0.7	1.5	4.1	1.0	1.8

TABLE III
Trace Elements in U.S G S. Standard Rocks (ppm)

	AGV-1	BCR-1	W-1	DTS-1	G-2	GSP-1	PCC-1
Ba (a)	1208	675	160	2.4	1870	1300	1.2
(b)	1140	630	156	<5	1970	1230	<5
Be (a)	3	1.7	0.8	–	2.6	1.5	–
(b)	2.6	2.1	0.9	<0.5	2.9	1.7	<0.5
Co (a)	14	38	47	133	5.5	6	112
(b)	18	36	41	128	7	9	109
Cr (a)	12	18	114	–	7	12	–
(b)	9	11	116	not analyzed	7	12	not analyzed
Cu (a)	60	18	110	7	12	33	11
(b)	59	15	122	7	12	31	11
U (a)	12	13	14	2	37	32	2
(b)	12	14	14	2.7	34	30	1.7
Ni (a)	18.5	16	76	2269	5	12.5	2339
(b)	12	<12	66	2420	<12	<12	2420
Pb (a)	35	18	8	14	31	51	13
(b)	40	16	<12	13	34	59	<12
Rb (a)	67	47	21	–	168	254	–
(b)	67	49	21	not analyzed	166	248	not analyzed
Sr (a)	657	330	190	0.35	479	233	0.41
(b)	698	363	194	<2	457	245	<2
V (a)	125	399	264	10	35	53	30
(b)	128	446	285	<15	42	62	23
Zn (a)	84	120	86	45	85	98	36
(b)	90	129	86	32	87	105	28

a Flanagan (8)
b This work

sorption offers improved accuracy and precision particularly for trace elements. This has been demonstrated by the analyses of U.S.G.S. standard rocks for 21 elements. The method could be extended to include other elements such as cadmium and gallium when they occur in higher concentration than those in the standard rocks.

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APPENDIX E

$HNO_3/HClO_4$ DIGESTION METHOD FOR TISSUE

(TETRA TECH 1986a)

11.0 PROCEDURE

11.1 Homogenize samples prior to analysis to ensure that a representative aliquot is taken. Any grinder or homogenizer that has been found to be free of contamination may be used. Samples should be ground wet to avoid losses of volatile elements (e.g., Hg, Se) during drying. The liquid associated with a sample after thawing should be retained as part of the sample.

11.2 Transfer the sample paste to a container suitable for storage. If not immediately analyzed, the samples should be frozen (-20° C) until required. Containers should be tightly sealed to prevent moisture loss or gain during storage.

11.3 Dry Weight Determination - if sample size permits and dry-weight concentrations are required, dry weight determinations may be performed as follows: transfer an aliquot of approximately 3 g (weighed to the nearest 0.1 g) to a pre-weighed dish. Allow the sample to dry in an oven at 105° C overnight, and determine the solid residue weight to the nearest 0.1 g. The percent total solids is calculated as:

$$T_S = [\text{dry residue wt (g)}]/[\text{west sample wt (g)}]$$

Dry-weight determinations should not be made at the cost of having insufficient sample for metals analysis. Significant decreases in the size of samples used for extraction will decrease attainable detection limits.

11.4 Accurately weigh representative aliquots of homogenized tissue to the nearest 0.1 mg. If sample size permits, approximately 5 g is required to maintain optimum detection limits. Transfer the weighed tissue to a pre-cleaned 125-mL Erlenmeyer flask equipped with an all-glass reflux cap. Analyze a sufficient number of reagent blanks, sample duplicates, analyte spikes, and certified reference materials concurrently.

11.5 Add 10.0 mL of concentrated nitric acid (ACS grade or better), replace cap and swirl. Allow flask to stand at room temperature for about 15 h in a dust-free ventilated environment. Periodically swirl the contents to help solubilize the tissue.

11.6 After 15 h, gently heat the flask to approximately 100° C - hold at this temperature for 1 h. Gradually increase the temperature in 50° C increments to a maximum of 250° C. Continue digesting until all tissue has been solubilized. This usually takes about 4 h. Do not rush the initial digestion as losses of volatile elements will likely occur. Once digestion is complete, cool flasks to room temperature and add 4.0 mL of perchloric acid.

> CAUTION: Perchloric acid is a strong oxidizing agent. The analyst must be fully aware of the precautions associated with its use. This procedure (i.e., use of perchloric acid at sub-fuming temperatures) has been safety performerd without a perchloric hod, but a perchloric hod is strongly recommended nonetheless. Laboratories that do not carefully monitor perchloric acid digestions will be endangered without perchloric hoods. Safety precautions and background information pertaining to perchloric acid can be found in Schilt (1979; Perchloric Acid and Perchlorates. G. Frederick Smith Chemical Company, Columbus, OH.).

11.7 Return flasks to the hotplate which has been cooled to about 200° C. Continue heating for 1 h, then increase plate temperature to 300° C. Hold at this temperture until all traces of nitric acid fumes have disappeared and the solutions have become clear. Do not overheat flasks or allow perchloric fumes (dense white) to appear. If perchloric fumes appear, reduce heat immediately. Remove the exracts when clear and cool them to room temperature.

11.8 When the digestion is complete, rinse the caps into the flasks and transfer the extract to a pre-cleaned 100-mL volumetric flask. Rinse the Erlenmeyer flask three times with DDW and combine with the extract previously added to the volumetric flask. Adjust the volume with DDW and transfer to a precleaned plastic bottle.

NOTE: Some elements are not as stable as others in solution and therefore should be analyzed first. Stability can be determined by daily analysis of the extracts. However, the following can be used as a guideline:

Sb, Pb, Hg, Se, and Ag - analyze within 1 day
As and Cd - analyze within 2 days
Cr, Cu, Ni and Zn - analyze within 1 wk
Be and Tl - to be determined.

APPENDIX F

APDC/MIBK EXTRACTION METHOD FOR SALT WATER

(GREENBURG ET AL. 1985)

303 B. Determination of Low Concentrations of Cadmium, Chromium, Cobalt, Copper, Iron, Lead, Manganese, Nickel, Silver, and Zinc by Chelation with Ammonium Pyrrolidine Dithiocarbamate (APDC) and Extraction into Methyl Isobutyl Ketone (MIBK)

1. Apparatus

a. Atomic absorption spectrometer and associated equipment: See Section 303.2.

b. Burner head, conventional. Consult manufacturer's operating manual for suggested burner head.

2. Reagents

a. Air: See 303A.2*a*.

b. Acetylene: See 303A.2*b*.

c. Metal-free water: See 303A.2*c*.

d. Methyl isobutyl ketone (MIBK), reagent grade. For trace analysis, purify MIBK by redistillation or by sub-boiling distillation.

e. Ammonium pyrrolidine dithiocarbamate (APDC) solution: Dissolve 4 g APDC in 100 mL water. If necessary, purify APDC with an equal volume of MIBK. Shake 30 s in a separatory funnel, let separate, and withdraw lower portion. Discard MIBK layer.

f. Nitric acid, HNO_3, conc, ultrapure.

g. Standard metal solutions: See 303A.2*l*.

h. Potassium permanganate solution, $KMnO_4$, 5% aqueous.

i. Sodium sulfate, Na_2SO_4, anhydrous.

j. Water-saturated MIBK: Mix one part

purified MIBK with one part water in a separatory funnel. Shake 30 s and let separate. Discard aqueous layer. Save MIBK layer.

k. Sodium azide solution: Dissolve 0.1 g NaN_3 in water and dilute to 100 mL.

3. Procedure

a. Instrument operation: See Section 303A.3*a*. After final adjusting of burner position, aspirate water-saturated MIBK into flame and gradually reduce fuel flow until flame is similar to that before aspiration of solvent.

b. Standardization: Select at least three standard metal solutions (prepared as in 303A.2*l*) to bracket expected sample metal concentration and to be, after extraction, in the optimum concentration range of the instrument. Adjust 100 mL of each standard and 100 mL of a metal-free water blank to pH 3 by adding 1*N* HNO_3 or 1*N* NaOH. For individual element extraction, use the following pH ranges to obtain optimum extraction efficiency:

Element	pH Range for Optimum Extraction
Ag	2–5 (complex unstable)
Cd	1–6
Co	2–10
Cr	3–9
Cu	0.1–8
Fe	2–5
Mn	2–4 (complex unstable)
Ni	2–4
Pb	0.1–6
Zn	2–6

NOTE For Ag and Pb extraction the optimum pH value is 2.3 ± 0.2. The Mn complex deteriorates rapidly, resulting in decreased instrument response. Unless Mn can be analyzed immediately after extraction, use another analytical procedure.

Transfer each standard solution and blank to individual 200-mL volumetric flasks, add 1 mL APDC solution, and shake to mix. Add 10 mL MIBK and shake vigorously for 30 s. (The maximum volume ratio of sample to MIBK is 40.) Let contents of each flask separate into aqueous and organic layers, then carefully add water down the side of each flask to bring the organic layer into the neck and accessible to the aspirating tube.

Aspirate organic extracts directly into the flame (zeroing instrument on a water-saturated MIBK blank) and record absorbance. With some instruments it may be necessary to convert percent absorption to absorbance by using a table generally provided by the manufacturer.

Prepare a calibration curve by plotting on linear graph paper absorbances of extracted standards against their concentrations before extraction.

c. Analysis of samples: Rinse atomizer by aspirating water-saturated MIBK. Aspirate organic extracts treated as above directly into the flame and record absorbances.

With the above extraction procedure only hexavalent chromium is measured. To determine total chromium, oxidize trivalent chromium to hexavalent chromium by bringing sample to a boil and adding sufficient $KMnO_4$ solution dropwise to give a persistent pink color while the solution is boiled for 10 min. Destroy excess $KMnO_4$ by adding 1 to 2 drops NaN_3 solution to the boiling solution, allowing 2 min for the reaction to proceed. If pink color persists, add 1 to 2 more drops NaN_3 solution and wait 2 min. Heat an additional 5 min. Cool, extract with MIBK, and aspirate.

During extraction, if an emulsion forms at the water-MIBK interface, add anhydrous Na_2SO_4 to obtain a homogeneous organic phase.

Determine recovery of silver whenever matrices change.

To avoid problems associated with instability of extracted metal complexes, determine metals immediately after extraction.

4. Calculations

Calculate the concentration of each metal ion in micrograms per liter by referring to the appropriate calibration curve.

APPENDIX G

DFAA INSTRUMENTAL AND SPECTROPHOTOMETRIC METHODS

(U.S. EPA 1979a)

ANTIMONY

Method 204.1 (Atomic absorption, direct aspiration)

STORET NO. Total 01097
Dissolved 01095
Suspended 01096

Optimum Concentration Range: 1–40 mg/l using a wavelength of 217.6 nm
Sensitivity: 0.5 mg/l
Detection Limit: 0.2 mg/l

Preparation of Standard Solution

1. Stock Solution: Carefully weigh 2.7426 g of antimony potassium tartrate (analytical reagent grade) and dissolve in deionized distilled water. Dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Sb (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.

Sample Preservation

1. For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.

Sample Preparation

1. The procedures for preparation of the sample as given in parts 4.1.1 through 4.1.4 of the Atomic Absorption Methods section of this manual have been found to be satisfactory.

Instrumental Parameters (General)

1. Antimony hollow cathode lamp
2. Wavelength: 217.6 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Fuel lean

Analysis Procedure

1. For analysis procedure and calculation, see "Direct Aspiration", part 9.1 of the Atomic Absorption Methods section of this manual.

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Interferences

1. In the presence of lead (1000 mg/1), a spectral interference may occur at the 217.6 nm resonance line. In this case the 231.1 nm antimony line should be used.
2. Increasing acid concentrations decrease antimony absorption. To avoid this effect, the acid concentration in the samples and in the standards should be matched.

Notes

1. Data to be entered into **STORET** must be reported as ug/1.
2. For concentrations of antimony below 0.35 mg/1, the furnace procedure (Method 204.2) is recommended.

Precision and Accuracy

1. In a single laboratory (EMSL), using a mixed industrial-domestic waste effluent at concentrations of 5.0 and 15 mg Sb/1, the standard deviations were ±0.08 and ±0.1, respectively. Recoveries at these levels were 96% and 97%, respectively.

ARSENIC

Method 206.3 (Atomic Absorption—gaseous hydride)

STORET NO. Total 01002
Dissolved 01000
Suspended 01001

1. Scope and Application
   1.1 The gaseous hydride method determines inorganic arsenic when present in concentrations at or above 2 *ug*/1. The method is applicable to drinking water and most fresh and saline waters in the absence of high concentrations of chromium, cobalt, copper, mercury, molybdenum, nickel and silver.
2. Summary of Method
   2.1 Arsenic in the sample is first reduced to the trivalent form using $SnCl_2$ and converted to arsine, AsH_3, using zinc metal. The gaseous hydride is swept into an argon-hydrogen flame of an atomic absorption spectrophotometer. The working range of the mehtod is 2–20 *ug*/1. The 193.7 nm wavelength line is used.
3. Comments
   3.1 In analyzing drinking water and most surface and ground waters, interferences are rarely encountered. Industrial waste samples should be spiked with a known amount of arsenic to establish adequate recovery.
   3.2 Organic forms of arsenic must be converted to inorganic compounds and organic matter must be oxidized before beginning the analysis. The oxidation procedure given in Method 206.5 (Standard Methods, 14th Edition, Method 404B, p. 285, Procedure 4.a) has been found suitable.
   3.3 For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.
   3.4 For quality control requirements and optional recommendations for use in drinking water analyses, see part 10 of the Atomic Absorption Methods section of this manual.
   3.5 Data to be entered into **STORET** must be reported as *ug*/1.
4. Precision and Accuracy
   4.1 Ten replicate solutions of o-arsenilic acid at the 5, 10 and 20 *ug*/1 level were analyzed by a single laboratory. Standard deviations were ±0.3, ±0.9 and ±1.1 with recoveries of 94, 93 and 85%, respectively. (Caldwell, J. S., Lishka, R. J., and McFarren, E. F., "Evaluation of a Low Cost Arsenic and Selenium Determination at Microgram per Liter Levels", JAWWA., vol 65, p 731, Nov., 1973.)

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5. References

5.1. Except for the perchloric acid step, the procedure to be used for this determination is found in: Standard Methods for the Examination of Water and Wastewater, 14th Edition, p159, Method 301A(VII),(1975)

ARSENIC

Method 206.4 (Spectrophotometric-SDDC)

STORET NO. 01002
Inorganic, Dissolved 00095
Inorganic, Total 00997
Inorganic, Suspended 00996

1. Scope and Application
   1.1 The silver diethyldithiocarbamate method determines inorganic arsenic when present in concentrations at or above 10 ug/1. The method is applicable to drinking water and most fresh and saline waters in the absence of. high concentrations of chromium, cobalt, copper, mercury, molybdenum, nickel, and silver. Domestic and industrial wastes may also be analyzed after digestion (see 3.3).
   1.2 Difficulties may be encountered with certain industrial waste materials containing volatile substances. High sulfur content of wastes may exceed removal capacity of the lead acetate scrubber.
2. Summary of Method
   2.1 Arsenic in the sample is reduced to arsine, AsH_3, in acid solution in a hydrogen generator. The arsine is passed through a scrubber to remove sulfide and is absorbed in a solution of silver diethyldithiocarbamate dissolved in pyridine. The red complex thus formed is measured in a spectrophotometer at 535 nm.
3. Comments
   3.1 In analyzing drinking water and most surface and ground waters, interferences are rarely encountered. Industrial waste samples should be spiked with a known amount of arsenic to establish adequate recovery.
   3.2 It is essential that the system be airtight during evolution of the arsine, to avoid losses.
   3.3 If concentration of the sample and/or oxidation of any organic matter is required, refer to Method 206.5. [Standard Methods, 14th Edition, Method 404B, p. 284, Procedure 4.a (1975)]. For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.
      3.3.1 Since nitric acid gives a negative interference in this test, use sulfuric acid as a preservative if only inorganic arsenic is being measured.
   3.4 1-Ephedrine in chloroform has been found to be a suitable solvent for silver diethyldithiocarbamate if the analyst finds the odor of pyridine objectionable [Anal. Chem. 45, 1786 (1973)].
   3.5 For quality control requirements and optional recommendations for use in drinking water analyses, see part 10 of the Atomic Absorption Methods section of this manual.

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4. Precision and Accuracy

4.1 In a round-robin study reported by Standard Methods a synthetic unknown sample containing 40 ug/l, as As, with other metals was analyzed in 46 laboratories. Relative standard deviation was ±13.8% and relative error was 0%.

5. Reference

5.1 The procedure to be used for this determination is found in:
Standard Methods for the Examination of Water and Wastewater, 14th Edition, p. 283, Method 404A (1975).

ARSENIC

Method 206.5 (Sample Digestion Prior to Total Arsenic Analysis by Silver Diethyldithiocarbamate or Hydride Procedures)

1. Scope and Application:
   1.1 Both the silver diethyldithiocarbamate spectrophotometric method and the AA hydride procedure measure inorganic arsenic. Therefore, if either of these procedures are being employed for the purpose of measuring total arsenic (inorganic plus organic), all organically bound arsenic must first be converted to an inorganic form prior to the analytical determination. This may be accomplished with H_2SO_4–HNO_3.
2. Procedure
   2.1 To a suitable sample containing from 2 to 30 *ug* of arsenic, add 7 ml (1 + 1) H_2SO_4 and 5 ml conc HNO_3. Evaporate the sample to SO_3 fumes. Caution: If the sample chars, stop the digestion immediately, cool and add additional conc HNO_3. Continue digestion adding additional conc HNO_3 as necessary.
   2.2 If the sample remains colorless, or straw-yellow during evolution of SO_3 fumes, the digestion is complete.
   2.3 Cool the digested sample, add about 25 ml distilled water, and again evaporate to SO_3 fumes to expel oxides of nitrogen.
   2.4 The sample is now ready for analysis using either the hydride or spectrophotometric procedure.
3. Interferences
   3.1 All traces of nitric acid must be removed before either the spectrophotometric or the hydride procedures are applied. Oxides of nitrogen should be expelled by taking the sample to fumes of SO_3.
4. Notes
   4.1 The digestion step may be carried out in a flask on a hot-plate or in a Kjeldahl apparatus. This digestion step may also be used, in effect, to concentrate the sample, inasmuch as any size volume may be processed.

Bibliography

1. Standard Methods for the Examination of Water and Wastewater, p285, method 404B, step 4a, 14th Edition (1975).

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CADMIUM

Method 213.1 (Atomic Absorption, direct aspiration)

STORET NO. Total 01027
Dissolved 01025
Suspended 01026

Optimum Concentration Range: 0.05–2 mg/l using a wavelength of 228.8 nm
Sensitivity: 0.025 mg/l
Detection Limit: 0.005 mg/l

Preparation of Standard Solution

1. Stock Solution: Carefully weigh 2.282 g of cadmium sulfate ($3CdSO_4 \cdot 8H_2O$, analytical reagent grade) and dissolve in deionized distilled water. 1 ml = 1 mg Cd (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.

Sample Preservation

1. For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.

Sample Preparation

1. The procedures for preparation of the sample as given in parts 4.1.1 through 4.1.4 of the Atomic Absorption Methods section of this manual have been found to be satisfactory.

Instrumental Parameters (General)

1. Cadmium hollow cathode lamp
2. Wavelength: 228.8 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

Analysis Procedure

1. For analysis procedure and calculation, see "Direct Aspiration", part 9.1 of the Atomic Absorption Methods section of this manual.

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Notes

1. For levels of cadmium below 20 ug/1, either the Special Extraction Procedure given in Part 9.2 of the Atomic Absorption methods section as the furnace technique, Method 213.2 is recommended.
2. Data to be entered into STORET must be reported as ug/1.
3. For quality control requirements and optional recommendations for use in drinking water analyses, see part 10 of the Atomic Absorption Methods section of this manual.

Precision and Accuracy

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of EMSL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, copper, iron, manganese, lead and zinc were added to natural water samples. The statistical results for cadmium were as follows:

Number of Labs	True Values ug/liter	Mean Value ug/liter	Standard Deviation ug/liter	Accuracy as % Bias
74	71	70	21	-2.2
73	78	74	18	-5.7
63	14	16.8	11.0	19.8
68	18	18.3	10.3	1.9
55	1.4	3.3	5.0	135
51	2.8	2.9	2.8	4.7

COPPER

Method 220.1 (Atomic Absorption, direct aspiration)

STORET NO. Total 01042
Dissolved 01040
Suspended 01041

Optimum Concentration Range: 0.2–5 mg/l using a wavelength of 324.7 nm
Sensitivity: 0.1 mg/l
Detection Limit: 0.02 mg/l

Preparation of Standard Solution

1. Stock Solution: Carefully weigh 1.00 g of electrolyte copper (analytical reagent grade). Dissolve in 5 ml redistilled HNO_3 and make up to 1 liter with deionized distilled water. Final concentration is 1 mg Cu per ml (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.

Sample Preservation

1. For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.

Sample Preparation

1. The procedures for preparation of the sample as given in parts 4.1.1 thru 4.1.4 of the Atomic Absorption Methods section of this manual have been found to be satisfactory.

Instrumental Parameters (General)

1. Copper hollow cathode lamp
2. Wavelength: 324.7 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

Analysis Procedure

1. For analysis procedure and calculation, see "Direct Aspiration", part 9.1 of the Atomic Absorption Methods section of this manual.

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Notes

1. For levels of copper below 50 *ug*/1, either the Special Extraction Procedure, given in part 9.2 of the Atomic Absorption Methods section or the furnace technique, Method 220.2, is recommended.
2. Numerous absorption lines are available for the determination of copper. By selecting a suitable absorption wavelength, copper samples may be analyzed over a very wide range of concentration. The following lines may be used:
   327.4 nm Relative Sensitivity 2
   216.5 nm Relative Sensitivity 7
   222.5 nm Relative Sensitivity 20
3. Data to be entered into STORET must be reported as *ug*/1.
4. The 2,9-dimethyl-1, 10-phenanthroline colorimetric method may also be used (Standard Methods, 14th Edition, p. 196).

Precision and Accuracy

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of EMSL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, copper, iron, manganese, lead and zinc were added to natural water samples. The statistical results for copper were as follows:

Number of Labs	True Values *ug*/liter	Mean Value *ug*/liter	Standard Deviation *ug*/liter	Accuracy as % Bias
91	302	305	56	0.9
92	332	324	56	-2.4
86	60	64	23	7.0
84	75	76	22	1.3
66	7.5	9.7	6.1	29.7
66	12.0	13.9	9.7	15.5

IRON
Method 236.1 (Atomic Absorption, direct aspiration)

STORET NO. Total 01045
Dissolved 01046
Suspended 01044

Optimum Concentration Range: 0.3–5 mg/l using a wavelength of 248.3 nm
Sensitivity: 0.12 mg/l
Detection Limit: 0.03 mg/l

Preparation of Standard Solution

1. Stock Solution: Carefully weigh 1.000 g of pure iron wire (analytical reagent grade) and dissolve in 5 ml redistilled HNO_3, warming if necessary. When solution is complete make up to 1 liter with deionized distilled water. 1 ml = 1 mg Fe (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.

Sample Preservation

1. For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.

Sample Preparation

1. The procedures for preparation of the sample as given in parts 4.1.1 thru 4.1.4 of the Atomic Absorption Methods section of this manual have been found to be satisfactory.

Instrumental Parameters (General)

1. Iron hollow cathode lamp
2. Wavelength: 248.3 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

Analysis Procedure

1. For analysis procedure and calculation, see "Direct Aspiration", part 9.1 of the Atomic Absorption Methods section of this manual.

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Notes

1. The following lines may also be used:
   248.8 nm Relative Sensitivity 2
   271.9 nm Relative Sensitivity 4
   302.1 nm Relative Sensitivity 5
   252.7 nm Relative Sensitivity 6
   372.0 nm Relative Sensitivity 10
2. Data to be reported into STORET must be reported as ug/1.
3. The 1,10-phenanthroline colorimetric method may also be used (Standard Methods, 14th Edition, p. 208).
4. For concentrations of iron below 0.05 mg/1, either the Special Extraction Procedure given in part 9.2 of the Atomic Absorption Methods section or the furnace procedure, Method 236.2, is recommended.

Precision and Accuracy

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of EMSL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, copper, iron, manganese, lead and zinc were added to natural water samples. The statistical results for iron were as follows:

Number Of Labs	True Values ug/liter	Mean Value ug/liter	Standard Deviation ug/liter	Accuracy as % Bias
82	840	855	173	1.8
85	700	680	178	-2.8
78	350	348	131	-0.5
79	438	435	183	-0.7
57	24	58	69	141
54	10	48	69	382

LEAD

Method 239.1 (Atomic Absorption, direct aspiration)

STORET NO. Total 01051
Dissolved 01049
Suspended 01050

Optimum Concentration Range: 1–20 mg/l using a wavelength of 283.3 nm
Sensitivity: 0.5 mg/l
Detection Limit: 0.1 mg/l

Preparation of Standard Solution

1. Stock Solution: Carefully weigh 1.599 g of lead nitrate, $Pb(NO_3)_2$ (analytical reagent grade), and dissolve in deionized distilled water. When solution is complete acidify with 10 ml redistilled HNO_3 and dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Pb (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.

Sample Preservation

1. For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.

Sample Preparation

1. The procedures for preparation of the sample as given in parts 4.1.1 thru 4.1.4 of the Atomic Absorption Methods section of this manual have been found to be satisfactory.

Instrumental Parameters (General)

1. Lead hollow cathode lamp
2. Wavelength: 283.3 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

Analysis Procedure

1. For analysis procedure and calculation, see "Direct Aspiration", part 9.1 of the Atomic Absorption Methods section of this manual.

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Notes

1. The analysis of this metal is exceptionally sensitive to turbulence and absorption bands in the flame. Therefore, some care should be taken to position the light beam in the most stable, center portion of the flame. To do this, first adjust the burner to maximize the absorbance reading with a lead standard. Then, aspirate a water blank and make minute adjustments in the burner alignment to minimize the signal.
2. For levels of lead below 200 *ug*/1, either the Special Extraction Procedure given in part 9.2 of the Atomic Absorption Methods section or the furnace technique, Method 239.2, is recommended.
3. The following lines may also be used:
   217.0 nm Relative Sensitivity 0.4
   261.4 nm Relative Sensitivity 10
4. For quality control requirements and optional recommendations for use in drinking water analyses, see part 10 of the Atomic Absorption Methods section of this manual.
5. Data to be entered into **STORET** must be reported as *ug*/1.

Precision and Accuracy

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of EMSL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, copper, iron, manganese, lead and zinc were added to natural water samples. The statistical results for lead were as follows:

Number of Labs	True Values *ug*/liter	Mean Value *ug*/liter	Standard Deviation *ug*/liter	Accuracy as % Bias
74	367	377	128	2.9
74	334	340	111	1.8
64	101	101	46	-0.2
64	84	85	40	1.1
61	37	41	25	9.6
60	25	31	22	25.7

MANGANESE
Method 243.1 (Atomic Absorption, direct aspiration)

STORET NO. Total 01055
Dissolved 01056
Suspended 01054

Optimum Concentration Range: 0.1–3 mg/l using a wavelength of 279.5 nm
Sensitivity: 0.05 mg/l
Detection Limit: 0.01 mg/l

Preparation of Standard Solution

1. Stock Solution: Carefully weigh 1.000 g of manganese metal (analytical reagent grade) and dissolve in 10 ml of redistilled HNO_3. When solution is complete, dilute to 1 liter with 1% (V/V) HCl. 1 ml = 1 mg Mn (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.

Sample Preservation

1. For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.

Sample Preparation

1. The procedures for preparation of the sample as given in parts 4.1.1 thru 4.1.4 of the Atomic Absorption Methods section of this manual have been found to be satisfactory.

Instrumental Parameters (General)

1. Manganese hollow cathode lamp
2. Wavelength: 279.5 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

Analysis Procedure

1. For analysis procedure and calculation, see "Direct Aspiration", part 9.1 of the Atomic Absorption Methods section of this manual.

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Notes

1. For levels of manganese below 25 *ug/l*, either the furnace procedure, Method 243.2, or the Special Extraction Procedure given in part 9.2 of the Atomic Absorption Methods section is recommended. The extraction is carried out at a pH of 4.5 to 5. The manganese chelate is very unstable and the analysis must be made without delay to prevent its resolution in the aqueous phase.
2. The following line may also be used:
   403.1 nm Relative Sensitivity 10.
3. Data to be entered into **STORET** must be reported as *ug/l*.
4. The persulfate colorimetric method may also be used (Standard Methods, 14th Edition, p 225).

Precision and Accuracy

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of EMSL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, copper, iron, manganese, lead and zinc were added to natural water samples. The statistical results for manganese were as follows:

Number of Labs	True Values *ug/liter*	Mean Value *ug/liter*	Standard Deviation *ug/liter*	Accuracy as % Bias
77	426	432	70	1.5
78	469	474	97	1.2
71	84	86	26	2.1
70	106	104	31	-2.1
55	11	21	27	93
55	17	21	20	22

NICKEL
Method 249.1 (Atomic Absorption, direct aspiration)

STORET NO. Total 01067
Dissolved 01065
Suspended 01066

Optimum Concentration Range: 0.3–5 mg/l using a wavelength of 232.0 nm
Sensitivity: 0.15 mg/l
Detection Limit: 0.04 mg/l

Preparation of Standard Solution

1. Stock Solution: Dissolve 4.953 g of nickel nitrate, $Ni(NO_3)_2 \cdot 6H_2O$ (analytical reagent grade) in deionized distilled water. Add 10 ml of conc. nitric acid and dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Ni (1000 mg/l).
2. Prepare dilutions of the stock nickel solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.

Sample Preservation

1. For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.

Sample Preparation

1. The procedures for preparation of the sample as given in parts 4.1.1 thru 4.1.4 of the Atomic Absorption Methods section of this manual have been found to be satisfactory.

Instrumental Parameters (General)

1. Nickel hollow cathode lamp
2. Wavelength: 232.0 nm
3, Fuel: Acetylene
4. Oxidant: Air
5. Type of Flame: Oxidizing

Analysis Procedure

1. For analysis procedure and calculation, see "Direct Aspiration", part 9.1 of the Atomic Absorption Methods section of this manual.

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Interferences

1. The 352.4 nm wavelength is less susceptible to spectral interference and may be used. The calibration curve is more linear at this wavelength; however, there is some loss of sensitivity.

Notes

1. For levels of nickel below 100 *ug*/1, either the Special Extraction Procedure, given in part 9.2 of the Atomic Absorption Methods section or the furnace technique, Method 249.2, is recommended.
2. Data to be entered into **STORET** must be reported as *ug*/1.
3. The heptoxime method may also be used (Standard Methods, 14th Edition, p 232).

Precision and Accuracy

1. In a single laboratory (EMSL), using a mixed industrial-domestic waste effluent at concentrations of 0.20, 1.0 and 5.0 mg Ni/1, the standard deviations were ±0.011, ±0.02 and ±0.04, respectively. Recoveries at these levels were 100%, 97% and 93%, respectively.

SILVER

Method 272.1 (Atomic Absorption, direct aspiration)

STORET NO. Total 01077
Dissolved 01075
Suspended 01076

Optimum Concentration Range: 0.1–4 mg/l using a wavelength of 328.1 nm
Sensitivity: 0.06 mg/l
Detection Limit: 0.01 mg/l

Preparation of Standard Solution

1. Stock Solution: Dissolve 1.575 g of $AgNO_3$ (analytical reagent grade) in deionized distilled water, add 10 ml conc. HNO_3 and make up to 1 liter. 1 ml = 1 mg Ag (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using nitric acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.
3. Iodine Solution, 1 N: Dissolve 20 grams of potassium iodide, KI (analytical reagent grade) in 50 ml of deionized distilled water, add 12.7 grams of iodine, I_2 (analytical reagent grade) and dilute to 100 ml. Store in a brown bottle.
4. Cyanogen Iodide (CNI) Solution: To 50 ml of deionized distilled water add 4.0 ml conc. NH_4OH, 6.5 grams KCN, and 5.0 ml of 1.0 N I_2 solution. Mix and dilute to 100 ml with deionized distilled water. Fresh solution should be prepared every two weeks.[1]

Sample Preservation

1. For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.

Sample Preparation

1. The procedures for preparation of the sample as given in parts 4.1.1 thru 4.1.3 of the Atomic Absorption Methods section of this manual have been found to be satisfactory; however, the residue must be taken up in dilute nitric acid rather than hydrochloric to prevent precipitation of AgCl.

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Instrumental Parameters (General)

1. Silver hollow cathode lamp
2. Wavelength: 328.1 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

Analysis Procedure

1. For the analysis procedure and the calculation, see "Direct Aspiration", part 9.1 of the Atomic Absorption Methods section of this manual.

Note:

1. For levels of silver below 30 *ug*/1, either the Special Extraction Procedure, given in part 9.2 of the Atomic Absorption Methods section or the furnace procedure, Method 272.2, is recommended.
2. Silver nitrate standards are light sensitive. Dilutions of the stock should be discarded after use as concentrations below 10 mg/1 are not stable over long periods of time.
3. If absorption to container walls or the formation of AgCl is suspected, make the sample basic using conc. NH_4OH and add 1 ml of (CNI) solution per 100 ml of sample. Mix the sample and allow to stand for 1 hour before proceeding with the analysis.[1]
4. The 338.2 nm wavelength may also be used. This has a relative sensitivity of 2.
5. Data to be entered into STORET must be reported as *ug*/1.

Precision and Accuracy

1. In a round-robin study reported by Standard Methods, a synthetic sample containing 50 *ug* Ag/1 was analyzed by 50 laboratories with a reported standard deviation of ±8.8 and a relative error 10.6%.

References

1. "The Use of Cyanogen Iodide (CNI) as a Stabilizing Agent for Silver in Photographic Processing Effluent Sample", Owerbach, Daniel, Photographic Technology Division, Eastman Kodak Company, Rochester, N.Y. 14650.
2. Standard Methods for Examination of Water and Wastewater, 14th Edition, p. 148, Method 301A.

ZINC

Method 289.1 (Atomic Absorption, direct aspiration)

STORET NO. Total 01092
Dissolved 01090
Suspended 01091

Optimum Concentration Range: 0.05–1 mg/l using a wavelength of 213.9 nm
Sensitivity: 0.02 mg/l
Detection Limit: 0.005 mg/l

Preparation of Standard Solution

1. Stock Solution: Carefully weigh 1.00 g of zinc metal (analytical reagent grade) and dissolve cautiously in 10 ml HNO_3. When solution is complete make up to 1 liter with deionized distilled water. 1 ml = 1 mg Zn (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The calibration standards should be prepared using the same type of acid and at the same concentration as will result in the sample to be analyzed either directly or after processing.

Sample Preservation

1. For sample handling and preservation, see part 4.1 of the Atomic Absorption Methods section of this manual.

Sample Preparation

1. The procedures for preparation of the sample as given in parts 4.1.1 thru 4.1.4 of the Atomic Absorption Methods section of this manual have been found to be satisfactory.

Instrumental Parameters

1. Zinc hollow cathode lamp
2. Wavelength: 213.9 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

Analysis Procedure

1. For the analysis procedure and the calculation, see "direct aspiration" part 9.1 of the Atomic Absorption Methods section of this manual.

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Notes

1. High levels of silicon may interfere.
2. The air-acetylene flame absorbs about 25% of the energy at the 213.9 nm line.
3. The sensitivity may be increased by the use of low-temperature flames.
4. Some sample container cap liners can be a source of zinc contamination. To circumvent or avoid this problem, the use of polypropylene caps is recommended.
5. The dithizone colorimetric method may also be used (Standard Methods, 14th Edition, p 265).
6. For concentrations of zinc below 0.01 mg/1, either the Special Extraction Procedure given in part 9.2 of the Atomic Absorption Methods section or the furnace procedure, Method 289.2, is recommended.
7. Data to entered into Storet must be reported as ug/1.

Precision and Accuracy

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of EMSL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, copper, iron, manganese, lead and zinc were added to natural water samples. The statistical results for zinc were as follows:

Number of Labs	True Values ug/liter	Mean Value ug/liter	Standard Deviation ug/liter	Accuracy as % Bias
86	281	284	97	1.2
89	310	308	114	-0.7
82	56	62	28	11.3
81	70	75	28	6.6
62	7	22	26	206
61	11	17	18	56.6

BENTHIC INFAUNA

SEDIMENT BIOASSAYS

FINAL REPORT
TC-3991-04

Puget Sound Estuary Program

RECOMMENDED PROTOCOLS FOR CONDUCTING LABORATORY BIOASSAYS ON PUGET SOUND SEDIMENTS

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May, 1986

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INTRODUCTION

This document presents recommended methods for conducting the following laboratory sediment bioassays in Puget Sound:

- Amphipod bioassay
- Bivalve larvae bioassay
- Anaphase aberration bioassay
- Microtox bioassay
  - Organic extraction
  - Saline extraction.

Each recommended method is based on the results of a workshop and written reviews by representatives from most organizations that fund or conduct environmental studies in Puget Sound (Table 1). The purpose of developing these recommended protocols is to encourage all Puget Sound investigators conducting monitoring programs, baseline surveys, and intensive investigations to use standardized methods whenever possible. If this goal is achieved, most data collected in the Sound will be directly comparable and thereby capable of being integrated into a sound-wide database. Such a database is necessary for developing and maintaining a comprehensive water quality management program for Puget Sound.

The sediment bioassays considered in this document are the tests used most frequently by a variety of Puget Sound investigators. However, other bioassays have been used or are currently being developed for use in Puget Sound. Several of these additional tests are described in the final section of this document (see Other Promising Techniques).

Each recommended protocol describes the use and limitations of the respective variable; the field collection and processing methods; and the laboratory analytical, quality assurance/quality control (QA/QC), and data reporting procedures. In developing each protocol, it was recognized that the field of sediment bioassays is relatively new and is expanding rapidly. The loose-leaf format of this document will allow modification of the recommended protocols, if necessary, and inclusion of additional protocols.

Although the following protocols are recommended for most studies conducted in Puget Sound, departures from these methods may be necessary to meet the special requirements of individual projects. If such departures are made, however, the funding agency or investigator should be aware that the resulting data may not be comparable with most other data of that kind.

TABLE 1. CONTRIBUTORS TO THE SEDIMENT BIOASSAY PROTOCOLS

John Armstrong[a]	U.S. EPA
Richard Batiuk	U.S. EPA
Scott Becker[a]	Tetra Tech
Alice Benedict[a]	NOAA
Jim Buckley[a]	Metro
Rick Cardwell[a]	Envirosphere Co.
Gary Chapman	U.S. EPA
Peter Chapman[a,b]	EVS Consultants
Joe Cummins[a]	U.S. EPA
Paul Dinnel	Univ. of Washington
Bruce Duncan[a]	U.S. EPA
Jim Fisher[a]	Weyerhaeuser Co.
Burt Hamner[a]	U.S. COE
Dave Jamison[a]	Wash. Dept. of Natural Resources
Dave Kendall[a]	U.S. COE
Dick Kocan[a]	Univ. of Washington
Mary Lou Mills[a]	Wash. Dept. of Fisheries
Phillip Oshida	U.S. EPA
Diane Robbins	Invert Aid
Pat Romberg[a]	Metro
Brian Ross	U.S. EPA
Norman Rubinstein	U.S. EPA
Mike Schiewe[a]	NOAA
Margaret Stinson[a]	Wash. Dept. of Ecology
Jerry Stober[a]	Univ. of Washington
John Strand[a]	Battelle Northwest
Rick Swartz	U.S. EPA
Dave Terpening[a]	U.S. EPA
Jim Thornton[a]	Wash. Dept. of Ecology
Milton Tunzi	U.S. EPA
Don Weitkamp[a]	Parametrix
Ron Westley[a]	Wash. Dept. of Fisheries
Les Williams[a]	Tetra Tech
Jack Word[a]	Evans-Hamilton

[a] Attended the workshop held on October 22, 1985.

[b] Workshop moderator.

In some instances, data collected using different methods may be compared if the methods are intercalibrated adequately.

Before protocols for specific bioassays are described, sections are presented on 1) the criteria used to identify the tests considered in this document, 2) protocols for field collection of surficial test sediments, and 3) general QA/QC procedures that apply to all sediment bioassays.

BIOASSAY SELECTION

A large number of sediment bioassays have been developed and used in recent years (Table 2). Many of these tests have been used to evaluate the toxicity of Puget Sound sediments. Eight tests (Table 3) were selected for further consideration based on the following criteria:

- Sensitivity - each test has detected biological effects in a variety of sediments
- Usage - each test has been used in more than one study in Puget Sound.

Of the eight sediment bioassays selected for consideration, four were identified as suitable for general application in Puget Sound for reasons outlined in Table 3. These four tests are categorized in Table 4 in terms of kind of test and kind of effect measured. The other tests identified in Table 3, together with some of the additonal tests mentioned in Table 2, are considered as other promising techniques at the end of this chapter.

TABLE 2. SUMMARY OF LABORATORY BIOASSAY TESTS WITH MARINE SEDIMENTS

Taxon	Investigators
ACUTE LETHAL BIOASSAYS	
Phytoplankton:	
two species test	Wurster (1982)
Annelid worms:	
* *Monopylephorus cuticulatus*	Chapman et al. (1982a)
* *Glycinde picta*	Swartz et al. (1979)
Crustaceans:	
(Copepods)	
* *Acartia tonsa*	Shuba et al. (1978)
* *Tigriopus* sp.	Shuba et al. (1978)
(Amphipods)	
* *Eogammarus confervicolus*	Chapman et al. (1982a)
* *Eohaustorius washingtonianus*	Ott et al. (in prep.)
* *Grandifoxus grandis*	Pierson et al. (1983)
T* *Rhepoxynius abronius*	Swartz et al. (1979; 1981; 1982; 1985a,b); Ott et al. (in prep.); Chapman et al. (1982a,b; 1984); Chapman and Fink (1983); Chapman and Barlow (1984); Malins et al. (1985); Tetra Tech (1985); Battelle (1986)
(Cumaceans)	
several species	Swartz et al. (1979)
(Shrimp)	
* *Palaemonetes pugio*	Shuba et al. (1978)

TABLE 2. (Continued)

Bivalve Molluscs:		
*	Macoma inquinata	Swartz et al. (1979)
*	Protothaca staminea	Swartz et al. (1979)
*	Rangia cuneata	Shuba et al. (1978)
	Mya arenaria	Tsai et al. (1979)
Fish:		
*	Gasterosteus aculeatus (threespine stickleback)	Chapman et al. (1982a)
*	Oncorhynchus kisutch (coho salmon)	Legore and DesVoigne (1973)
*	Oncorhynchus tshawytscha (chinook salmon)	Pierson et al. (1983)
	Fundulus heteroclitus (mummichog)	Tsai et al. (1979); Hargis et al. (1984)
	Leiostomus xanthurus (spot)	Tsai et al. (1979); Hargis et al. (1984)
SUBLETHAL BIOASSAYS		
T*	Respiration rate of the oligochaete worm, Monopylephorus cuticulatus	Chapman (in press); Chapman et al. (1982a,b; 1984; 1985); Chapman and Fink (1983)
T*	Oyster larvae, Crassostrea gigas, partial life-cycle bioassay	Schink et al. (1974); Cummins et al. (1976); Chapman and Morgan (1983); Chapman et al. (1983; 1985); Pierson et al. (1983); Malins et al. (1985); Tetra Tech (1985); Battelle (1986)
T*	Surf smelt, Hypomesus pretiosus pretiosus, partial life-cycle bioassay	Chapman et al. (1983); Malins et al. (1985)
*	Full life-cycle bioassay with the polychaete worm, Capitella capitata	Chapman et al. (1983); Chapman and Fink (1984)

TABLE 2. (Continued)

*	Histopathological measures of toxicity to English sole, *Parophrys vetulus*, with 3 mo. sediment exposure	McCain et al. (1982)
	Phytotoxicity to marsh plant *Cyperus esculentus*	Lee et al. (1982)
T*	Sea urchin fertilization assay with *Strongylocentrotus purpuratus*	Ross et al. (1984) Malins et al. (1985)
	Full life-cycle bioassays with nematodes *Chromadorina germanica* and *Diploeaimella punicea*	Tietjen and Lee (1984)
T*	Partial life-cycle bioassays with the copepod *Tigriopus californicus*	Misitano (1983); Malins et al. (1985)
CYTOTOXIC/GENOTOXIC BIOASSAYS		
T*	Mitotic abnormalities (anaphase aberrations) and cell proliferation in vitro	Chapman et al. (1982a,b; 1983; 1984; 1985); Landolt and Kocan (1984a,b)
MICROBIAL ACTIVITY BIOASSAYS		
T*	Microtox, *Photobacterium phosphoreum*, bacterial luminescence	Williams et al. (in press); Schiewe et al. (1985); Malins et al. (1985)

* Indicates used in Puget Sound.

T Sensitive method used in more than one study.

TABLE 3. APPLICABILITY AND RELEVANCE OF SENSITIVE LABORATORY SEDIMENT BIOASSAY TESTS USED IN MORE THAN ONE STUDY

Bioassay	Type[a]	Acceptable for Immediate Use in Puget Sound?	Comment
Amphipod 10-day test (*Rhepoxynius abronius*)	L,S	Yes	Simple test, reproducible, of ecological relevance, applicability verified in numerous studies, extensive usage to date in Puget Sound
Oligochaete respiration rate measurements (*Monopylephorus cuticulatus*)	S	No	Provides useful information, but requires specialized equipment and expertise not generally available (Chapman et al. 1985)
Bivalve larvae 48-h test (*Crassostrea gigas*, *Mytilus edulis*)	L,S	Yes	Simple test, indicator of toxicity, relevance and applicability verified in several independent studies, extensive usage to date in Puget Sound
Echinoderm sperm test	L,S	No	Simple test, can be conducted year-round by using different echinoderm species, but interfering factors cannot yet be eliminated (Ross et al. 1984; Malins et al. 1985; Dinnel and Stober 1985)
Surf smelt partial life-cycle test (*Hypomesus pretiosus pretiosus*)	L,S,C	No	Smelt eggs not available year-round, results confounded by excessive natural variability (Chapman et al. 1983, 1985)
Copepod partial life-cycle test (*Tigriopus californicus*)	L,S,C	No	Simple test, can be conducted year-round, but procedure has not been fully validated (D. Misitano, NMFS, pers. comm.; P. Chapman, E.V.S. Consultants, unpub. data)

TABLE 3. (Continued)

In vitro anaphase aberration tests with rainbow trout gonad cells (*Salmo gairdneri*)	G	Yes	One of the few methods to measure possible genotoxic/cytotoxic effects, moderate usage to date in Puget Sound, requires a higher level of expertise than other tests but can be conducted by more than one laboratory in Puget Sound
Microtox, bacterial luminescence bioassay (*Photobacterium phosphoreum*)	S	Yes	One of the few methods to measure possible effects on bacteria, shows good promise, moderate usage to date in Puget Sound

[a] L = Lethal; S = Sublethal; C = Chronic; G = Genotoxic.

TABLE 4. CATEGORIZATION OF SELECTED SEDIMENT BIOASSAYS BY KIND OF TEST AND KIND OF EFFECT

Kind of Test	Kind of Effect[a]		
	Lethal	Sublethal	Genotoxic
Solid and liquid phase:			
Rhepoxynius abronius	X	X	
Bivalve larvae	X	X	
Extracts:			
Microtox		X	
Anaphase aberrations			X

[a] None of these tests measures chronic effects (i.e., defined as longer term than 10 days, involving partial life-cycle testing).

FIELD COLLECTION OF SUBTIDAL SURFICIAL SEDIMENTS

This section describes the protocols required to collect an acceptable subtidal surficial sediment sample for subsequent analysis by a laboratory sediment bioassay. This subject has generally been neglected in the past and sampling crews have been given relatively wide latitude in deciding how to collect samples. However, because sample collection procedures influence the results of all subsequent laboratory and data analyses, it is critical that samples be collected using acceptable and standardized techniques.

DESIGN OF SAMPLER

In Puget Sound, the most common sampling device for subtidal surficial sediments is the modified van Veen bottom grab. However, a variety of coring devices (e.g., box corer, Kasten corer) are also used. The primary criterion for an adequate sampler is that it consistently collect undisturbed samples to the required depth below the sediment surface without contaminating the samples. An additional criterion is that the sampler can be handled properly on board the survey vessel. An otherwise acceptable sampler may yield inadequate sediment samples if it is too large, heavy, or awkward to be handled properly.

Collection of undisturbed sediment requires that the sampler:

- Create a minimal bow wake 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.

Most modified van Veen grabs have open upper faces that are fitted with rubber flaps. Upon descent, the flaps are forced open to minimize the bow wake, whereas upon ascent, the flaps are forced closed to prevent sample winnowing. Some box corers have solid flaps that are clipped open upon descent, and snap shut after the corer is triggered. Although most samplers seal adequately when purchased, the wear and tear of repeated field use eventually reduces this sealing ability. A sampler should therefore be monitored constantly for sample leakage. If unacceptable leakage occurs, the sampler should be repaired or replaced. If a sampler is to be borrowed or leased for a project, its sealing ability should be confirmed prior

to sampling. Also, it is prudent to have a backup sampler on board the survey vessel if the primary sampler begins leaking during a cruise.

The required penetration depth below the sediment surface is a function of the desired sample depth (see Penetration Depth). Generally, it is better to penetrate below the desired sample depth to minimize sample disturbance when the sampling device closes. Penetration depth of most sampling devices varies with sediment character; it is greatest in fine sediments and least in coarse sediments. Sampling devices generally rely upon either gravity or a piston mechanism to penetrate the sediment. In both cases, penetration depth can be modified by adding or subtracting weight from the samplers. Thus, it is optimal to use a sampler that has a means of weight adjustment. If a sampler cannot consistently achieve the desired penetration depth, an alternate device should be used.

Once the sampler is secured on board the survey vessel, it is essential that the surface of the sample be made accessible without disturbing the sample. Generally, samplers have hinged flaps on their upper face for this purpose. The openings in the upper face of the sampler should be large enough to allow easy subsampling of the sediment surface. If an opening is too small, the sample may be disturbed as the field member struggles to take a subsample.

PENETRATION DEPTH

For characterizing the toxicity of surficial sediments in Puget Sound, it is recommended that the upper 2 cm of sediment be evaluated. When collecting the upper 2 cm of sediment, it is recommended that a minimum penetration depth of 4-5 cm be achieved for each acceptable sample.

Although the 2-cm specification is arbitrary, it will ensure that:

- Relatively recent sediments are sampled
- Adequate volumes of sediment can be obtained readily for laboratory analyses
- Data from different studies (historical or ongoing) can be compared validly.

Sampling depths other than 2 cm may be appropriate for specific purposes. For example, when toxicity determinations are made for marine dredge disposal permitting, sediments collected from depths as great as several meters may be tested. It should be remembered, however, that if a sampling depth other than 2 cm is used, comparisons with data from 2-cm deep samples may be questionable.

OPERATION OF SAMPLER

The sampling device should be attached to the hydrowire using a ball-bearing swivel. The swivel will minimize the twisting forces on the sampler during deployment and ensure that proper contact is made with the bottom. For safety, the hydrowire, swivel, and all shackles should have a load capacity at least three times greater than the weight of a full sampler.

The sampler should be lowered through the water column at a controlled speed of approximately 1 ft per sec. Under no circumstances should the sampler be allowed to "free fall" to the bottom, as this may result in premature triggering, an excessive bow wake, or improper orientation upon contact with the bottom. The sampler should contact the bottom gently, and only its weight or piston mechanism should be used to force it into the sediment.

After the sediment sample is taken, the sampler should be raised slowly off the bottom and then retrieved at a controlled speed of approximately 1 ft per sec. Before the sampler breaks the water surface, the survey vessel should head into the waves (if present) to minimize vessel rolling. This maneuver will minimize swinging of the sampler after it breaks the water surface. If excessive swinging occurs or if the sampler strikes the vessel during retrieval, extra attention should be paid to evaluating sample disturbance when judging sample acceptability.

The sampler should be secured immediately after it is brought on board the survey vessel. If the sampler tips or slides around before being secured, extra attention should be paid to evaluating sample disturbance.

SAMPLE ACCEPTABILITY CRITERIA

After the sampler is secured on deck, the sediment sample should be inspected carefully before being accepted. The following acceptability criteria should be satisfied:

- The sampler is not overfilled with sample so that the sediment surface is pressed against the top of the sampler
- Overlying water is present (indicates minimal leakage)
- The overlying water is not excessively turbid (indicates minimal sample disturbance)
- The sediment surface is relatively flat (indicates minimal disturbance or winnowing)
- The desired penetration depth is achieved (i.e., 4-5 cm for a 2-cm deep surficial sample).

If a sample does not meet any one of these criteria, it should be rejected.

SAMPLE COLLECTION

After a sample is judged acceptable, the following observations should be noted on the field log sheet:

- Station location
- Depth
- Gross characteristics of the surficial sediment
  - Texture
  - Color
  - Biological structures (e.g., shells, tubes, macrophytes)
  - Presence of debris (e.g., wood chips, wood fibers, human artifacts)
  - Presence of oily sheen
  - Odor (e.g., hydrogen sulfide, oil, creosote)
- Gross characteristics of the vertical profile
  - Changes in sediment characteristics
  - Presence and depth of redox potential discontinuity (rpd) layer
- Penetration depth
- Comments relative to sample quality
  - Leakage
  - Winnowing
  - Disturbance.

Before subsamples of the surficial sediments are taken, the overlying water must be removed. The preferred method of removing this water is by slowly siphoning it off near one side of the sampler. Methods such as decanting the water or slightly cracking the grab to let the water run out are not recommended, as they may result in unacceptable disturbance or loss of fine-grained surficial sediment and organic matter.

Once the overlying water has been removed, the surficial sediment can be subsampled. It is recommended that subsamples be taken using a flat scoop shaped like a coal shovel. The shoulders of the scoop should be 2 cm high. This device will allow a relatively large subsample to be taken accurately to a depth of 2 cm. Coring devices are not recommended because generally they collect small amounts of surficial sediment, and therefore require repeated extractions to obtain a sufficient volume of material for analysis of conventional sediment variables. A curved scoop

is not recommended because it does not sample a uniform depth. Because accurate and consistent subsampling requires practice, it is advisable that an experienced person perform this task.

Finally, sample contamination during collection must be avoided. All sampling equipment (e.g., scoops, containers) should be made of non-contaminating material, and should be cleaned appropriately before use. It is recommended that all objects coming in contact with the sample be made of glass, stainless steel, or TFE (i.e., tetrafluoroethylene; e.g., Teflon). To avoid contamination, all sampling equipment should be washed thoroughly with pesticide-grade methylene chloride prior to initial use and between use for each sample.

SAMPLE HOMOGENIZATION

Both compositing of samples for chemistry and bioassay testing, and subsampling bioassay samples for different types of testing require thorough mixing (homogenization) of the initial sample. This is particularly important when the contents of several samples are required to provide sufficient material for testing.

Compositing and homogenization can be accomplished by transferring sediment to a solvent-rinsed glass or stainless steel bowl and thoroughly homogenizing by stirring with a stainless steel spoon and spatula until textural and color homogeneity are achieved. The bowl and all utensils should be solvent-rinsed between composites, and kept covered with aluminum foil to prevent airborne or other contamination.

CONCURRENT COLLECTION OF SEDIMENT CHEMISTRY AND BIOASSAY SAMPLES

If sediment chemistry samples are being collected along with sediment bioassay samples they should be collected from the same sample. Specifically, composites of several samples or of individual samples should be combined and mixed before aliquots are removed for sediment chemistry and sediment bioassay determinations. Sample homogenization and collection of bioassay aliquots should be conducted so that chemical aliquots are not contaminated in the process. The protocols for collecting aliquots for analyses of organic compounds and metals should be consulted in the appropriate chapters of this notebook to ensure that collection procedures for bioassay aliquots are compatible with those recommended for chemical aliquots.

GENERAL QUALITY ASSURANCE/QUALITY CONTROL GUIDELINES

The following general QA/QC procedures apply to all sediment bioassays. QA/QC procedures specific to each bioassay for which protocols are detailed, and that differ from the following generic guidelines, are incorporated into individual bioassay protocols.

NEGATIVE CONTROLS

All bioassays must be conducted using well-established negative (clean) controls. For every test series with a particular organism, one bioassay test chamber or series of chambers must contain clean, inert material plus diluent seawater. The complete bioassay series must be repeated if more than 10 percent of the control animals die or show evidence of sublethal effects.

POSITIVE CONTROLS

All bioassays should be conducted using well-established positive (toxic) controls. These controls involve the use of reference toxicants. Reference toxicants are used to provide insight into mortalities or increased sensitivity that may occur as a result of disease, changes in tolerance/sensitivity, and loading density. Reference toxicants can also provide insight into nonlethal effects that occur due to acclimation, insensitivity, or stress tolerance developed in handling and bioassay. Accordingly, concurrent bioassays using a reference toxicant should be implemented for each test series.

TEST ORGANISMS

Only healthy organisms of similar size and life history stage should be used in bioassays. Taxonomic identifications of bioassay organisms must be confirmed by a qualified taxonomist.

REFERENCE TEST SAMPLES

Control sediments generally are those from which test animals were collected. As such, physical and chemical sediment characteristics (e.g., grain size, organic content) may be very different from those of the test sediments. Where this is the case, one or more reference sediments should be added to the test series. Reference sediments should be collected from an area documented to be free from chemical contamination and should represent the range of important physical and chemical variables found in the test sediments. Data derived from such a sample, if in fact it is totally free of contamination, can be used to partition toxicant effects from unrelated effects such as those of sediment grain size.

BLIND TESTING

All treatment and bioassay containers should be randomized and testing should be conducted without laboratory personnel knowing sample identities. Replicates of each treatment should be assigned a code number during testing and randomized in the test sequence.

MAINTENANCE/MEASUREMENT OF WATER QUALITY

Bioassays involving exposure of organisms in aqueous media require that the media be uncontaminated and that proper water quality conditions be maintained to ensure the survival of the organisms, and to ensure that undue stress is not exerted on the organisms unrelated to the test sediments. At a minimum, the following variables must be measured at the beginning and termination of testing: salinity, dissolved oxygen, pH, and temperature.

STANDARD LABORATORY PROCEDURES

Standard laboratory procedures must be followed in all testing. These include proper documentation, proper cleaning, avoidance of contamination, and maintenance of appropriate test conditions. All unusual observations or deviations from established procedures must be recorded and reported.

AMPHIPOD SEDIMENT BIOASSAY

USE AND LIMITATIONS

The amphipod (Rhepoxynius abronius) sediment bioassay is used to characterize the toxicity of marine sediments. This bioassay may be used alone as a screening tool in broad-scale sediment surveys, in combination with sediment chemistry and in situ biological indices, and in laboratory experiments addressing a variety of sediment and water quality manipulations.

The amphipod bioassay is not appropriate for sediments that have an interstitial salinity of less than 15 ppt. In addition, the following constraints apply:

- An interstitial salinity of 25 ppt is necessary to ensure that there are no salinity effects. If interstitial salinities are between 15 and 24 ppt, they must be adjusted upward.

- Grain size may have an effect on the animals at extremes of fine and coarse material. If the clay content of the test sediments exceeds 50 percent or the gravel content exceeds 35 percent, controls for the effects of particle size distribution are recommended.

- As in all bioassays using natural populations, there is a possibility that relative sensitivity of the amphipods will vary with season or other factors. Accordingly, a positive control is recommended. This should comprise a 96-h LC50 measurement with a reference toxicant (e.g., $CdCl_2$ or NaPCP) conducted in the absence of sediment.

- Identification of R. abronius must be confirmed by a qualified taxonomist prior to initiation of the bioassay, and representative specimens should be preserved and archived for future reference.

- Predators generally are not a problem in the bioassay, but potential problems can be avoided by observation and reasonable care.

FIELD PROCEDURES

Collection

Test Animals--

Amphipods are obtained using benthic grabs (e.g., van Veen, Smith-McIntyre) or small dredges. If a dredge is used, a short haul (10 m) will minimize potential damage to the animals during collection. Approximately one third more animals than are required for the bioassay are collected. Surface and bottom seawater salinity and temperature are measured at the collection site. Sediment temperature is recorded from the first and last dredge sample. It is recommended that bioassays be conducted within 10 days of amphipod collection.

Sediment--

Both control and test sediment should be collected in solvent-cleaned glass jars having TFE-lined lids. Each jar should be filled completely to exclude air. A minimum sediment sample size of 0.25 L for each bioassay beaker is recommended for both kinds of sediment.

Processing

Test Animals--

Contents of the dredge are gently washed into a container using seawater of similar temperature and salinity to that at the collection depth. Samples that show evidence of contamination (e.g., oil sheen) are rejected. Amphipods will typically bury in the sediment and if necessary can be held in the containers for several hours (at the bottom temperature) prior to sieving. It is preferable to minimize the delay from collection to sieving. To avoid handling stress, each dredge sample is placed in a separate container. Animals are maintained and transported in clean coolers (with ice as necessary).

Sediment--

Both control and test sediment should be stored at 4° C in the dark. Holding time should not exceed 14 days.

LABORATORY PROCEDURES

The laboratory procedures are those described by Swartz et al. (1985) with the following changes incorporated:

- The salinity of the overlying water is increased to 28 ppt
- Reburial is omitted as a response variable

- A 1.0-mm screen is used to sieve out amphipods prior to initiation of testing
- Holding time for amphipods is standardized to between 4 and 10 days
- Sediment holding time prior to testing is set at a maximum of 14 days
- Additional details are provided concerning maintenance and transportation of amphipods, confirmation of taxonomic identifications, and freeing of amphipods trapped during testing by water surface tension
- A specific procedure for interstitial salinity adjustment is recommended
- A positive control is stipulated and data on 96-h LC50 values for two reference toxicants are provided.

Test Animals

Sieving--

A 1.0-mm sieve is used to remove R. abronius from sediment. Mature amphipods (3.0-5.0 mm total length) are used in the sediment bioassay. Gentle sieving is essential to reduce handling stress. The sieve is placed in a large tub filled with seawater at ambient collection site bottom salinity and temperature. The entire contents of each holding container, including water, are washed through the sieve using seawater pumped at low pressure through a fan spray nozzle. The sieve can be shaken gently, but the bottom of the screen must be beneath the water surface at all times. Material retained on the screen is washed into buckets for sorting. Large pieces of detritus and obvious predators are discarded. If there is a delay of more than 1 h before sorting begins, the buckets should contain enough sieved sediment to allow the amphipods to bury. The buckets must be kept at collection site temperature. Aeration may be necessary.

Sorting--

An aliquot of detritus or sediment containing amphipods is placed in a sorting tray. Healthy, active animals are removed with a bulb pipette (5-mm opening) and placed in 10-cm diameter finger bowls filled with 28 ppt seawater and a 2-cm deep layer of 0.5-mm sieved collection site sediment. Twenty amphipods are held in each bowl and enough bowls are prepared to provide at least one third more specimens than are required for the bioassay. For R. abronius, seawater temperature during sorting must not exceed 18° C. Filled finger bowls are submerged in holding tanks supplied with flowing

water or aeration where temperature and salinity approximate bioassay conditions. If temperature and salinity adjustments are necessary, they should be made gradually. Healthy R. abronius will remain in the finger bowl sediment and can be retrieved easily when the bioassay is set up. Amphipods should be acclimated to laboratory conditions for a minimum of 4 days and a maximum of 10 days before testing.

The identification of amphipods as R. abronius must be confirmed by a qualified taxonomist. In addition, representative specimens from each bioassay series should be preserved and archived for future reference.

Control Sediment

R. abronius typically inhabits well-sorted, fine sand. Suggestions for sieving and settling may have to be adjusted for other sediment types.

Approximately 0.25 L of control sediment is collected for each bioassay beaker. This sediment is sieved twice: first, to remove the test species and other macrobenthos and second, to adjust interstitial water salinity. The entire contents of one or more sediment samples, including water and suspended particulate matter, are sieved through a 0.5-mm screen without allowing overflow from the container. After the first sieving, the sediment is allowed to settle for at least 4 h. Overlying water is then decanted and the sediment resieved through a 0.5-mm screen into water of the bioassay salinity (28 ppt for R. abronius). Again, the sediment is allowed to settle for at least 4 h (preferably overnight), overlying water is decanted, and the control sediment is held at 4° C until the bioassay chambers are prepared.

Test Sediment

Approximately 0.25 L of test sediment are necessary for each bioassay beaker. The natural geochemical properties of test sediment collected from the field must be within the tolerance limits of the test species. R. abronius may be adversely affected by salinity stress if the interstitial water salinity is below 25 ppt. Interstitial salinities below 25 ppt may therefore require adjustment upwards. This species is tolerant of a broad range of sediment types. However, controls for the effects of particle size distribution are recommended if the clay content exceeds 50 percent or the gravel content exceeds 35 percent.

The R. abronius test requires a minimum water column salinity regime of 28 ppt. When the interstitial (i.e., pore water) salinity is below 25 ppt, it must be raised if this test is to be used. The following procedure is recommended in such cases. The interstitial salinity of the sediments is determined (e.g., by refractometer) and the sediments are placed in the bioassay chambers with overlying water of a salinity calculated to raise interstitial salinities to a minimum of 25 ppt. The sediments are then carefully and slowly stirred by hand with a clean glass rod for 1 min, and allowed to settle and equilibrate. The majority (approximately

75 percent) of the overlying water is then carefully decanted and the interstitial salinity in each chamber confirmed prior to bioassay initiation. The decant water is retained, salinity adjusted if necessary, and used as the overlying water in the bioassay. Sediments are mixed after adding the decant water. After the bioassays terminate, the interstitial salinities are reconfirmed.

Bioassay Seawater

Seawater used in the bioassay is maintained at a salinity of 28 ± 1 ppt and temperature of 15 $\pm 1^{\circ}$ C. If a series of experiments is planned, test temperature and salinity should be the same throughout the series. The bioassay seawater must be uncontaminated, which may necessitate collection of seawater at the amphipod collection site. The quantity of seawater required is dependent on sieving and holding needs and on the number of bioassay chambers.

Bioassay seawater is passed through a filter with 0.45-um pore diameter. If necessary, salinity is reduced by addition of deionized distilled water or raised by addition of clean oceanic water or reagent grade chemicals (ASTM Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians [E 729]). Seawater is prepared within 2 days of the bioassay and stored in covered, clean containers at the bioassay temperature.

Facilities and Equipment

The bioassay chamber is a standard 1-L glass beaker (10-cm internal diameter) covered with an 11.4-cm diameter glass watchglass. The beakers are placed in a shallow water bath or temperature-controlled room with overhead aeration source. Aeration to each beaker is provided through a 1-mL glass pipette that extends between the beaker spout and watchglass to a depth not closer than 2 cm from the sediment surface. Air is bubbled into the beakers at a rate that does not disturb the sediment. The bioassay temperature is maintained by either the water bath or room temperature control.

All glassware is cleaned by washing with laboratory detergent; rinsing in turn with distilled water, 10 percent nitric acid (HNO_3) or hydrochloric acid (HCl), and distilled water. Large plastic containers and plastic sieves used for preparation and storage of sediment and seawater are preconditioned initially by soaking for 24 h in seawater and rinsed after each use with clean seawater. They are used only for bioassays and stored in a clean room. Sieves and containers used to collect and store amphipods, seawater, and control sediment are kept separate from those used for test sediment.

Bioassay Procedure

Approximately 175 mL of test sediment are placed in the bottom of the 1-L bioassay chamber to create a 2-cm deep layer. Beakers are filled to 950 mL with 28 ppt seawater, covered with a watchglass, and placed in a 15° C water bath. Constant illumination is provided by overhead lights. Water in the beakers is aerated without disturbing the sediment surface. Twenty amphipods are placed in each beaker. The bioassay is terminated after 10 days of exposure.

The primary response criterion is survival after 10 days exposure to test or control sediment. The secondary response criterion is daily emergence of amphipods from toxic sediment.

Initiation--

The day before the bioassay is initiated, each test sediment sample is mixed within its storage container and an aliquot sufficient to make a 2-cm deep layer is added to a bioassay beaker. For replicate bioassay samples, the weight of sediment necessary to make a 2-cm deep layer (approximately 175 mL) in the first beaker is added to the other replicates. The same procedure applies to control sediment. Treatments are randomly assigned to prenumbered bioassay beakers.

The sediment aliquot in the beaker is settled by smoothing with a spoon, and bubbles are removed by tapping the beaker against the palm of the hand. A disk (attached to a string for removal) is placed on the sediment surface. This minimizes sediment disruption as bioassay seawater is added up to the 750-mL mark on the beakers. This disk is removed and rinsed in bioassay water between beakers and changed between treatments. The beakers are covered with watchglasses, put into the 15° C water bath or temperature-controlled room, and aerated. The beakers are allowed to equilibrate overnight to bioassay conditions. Normal room lighting is maintained continuously during the bioassay. If the experimental design requires monitoring of sediment chemistry (e.g., metals, total volatile solids, Eh), additional beakers must be set up for this purpose. Monitoring the quality of seawater overlying the sediment can be accomplished in the bioassay beakers without disturbing the sediment. Temperature is recorded from a thermometer maintained in a separate beaker containing control sediment and bioassay water but no amphipods.

On the day the bioassay is initiated, amphipods are distributed among all beakers so that each receives 20 individuals. It is usually not logistically possible to distribute amphipods to all beakers at the same time, so it necessary to select a portion of the beakers (as many as 15) to be processed together. The exact number of beakers to receive amphipods at one time is dependent on the size and design of the experiment. At least one replicate from each treatment, including control sediment, is processed at a time if possible. Otherwise, selection is random.

Amphipods are removed from the holding sediment using a 1.0-mm sieve, and then transferred to sorting trays. About one third more fingerbowls are removed from the holding tank at one time than are required for the number of beakers. This allows selection of active, healthy animals for the bioassay. Amphipods are removed from the sorting tray and sequentially distributed among clean 10-cm fingerbowls each containing 150 mL of bioassay seawater without sediment. The number of amphipods distributed to each fingerbowl is recounted by transferring them to a separate fingerbowl.

Amphipods are added to the bioassay beakers by placing a black plastic disk on the seawater surface and gently pouring the entire contents of the fingerbowl into the beaker. The fingerbowl is washed with bioassay water to remove adhering amphipods. The seawater level is brought up to 950 mL with bioassay water, and the disk is removed and rinsed between samples. Amphipods are allowed to bury in the sediment and any that are floating on the seawater surface are pushed down with the edge of the beaker cover. After 1 h, amphipods that have not buried are removed and replaced. Normally, less than 1 percent of the animals will fail to bury in 1 h.

Monitoring--

If samples for chemical analysis are desired, seawater and sediment samples can be taken from beakers at the initiation of the bioassay. A small quantity of seawater can be taken from beakers at the initiation of the bioassay, but chemistry beakers have to be sacrificed to obtain sediment samples. This is accomplished by siphoning the overlying seawater without disturbing the sediment surface and then taking appropriate sediment aliquots for chemical analyses. It is not necessary to add amphipods to chemistry beakers that are sacrificed at the initiation of the bioassay, but amphipods are added to those sacrificed later. Certain sediment and water quality variables (e.g., dissolved oxygen, pH, Eh) can be monitored by inserting analytical probes into the chemistry beakers.

During the course of the bioassay, certain observations are made daily. Temperature in the beaker set up for this purpose is monitored. Lighting and aeration systems are checked. Each beaker is carefully examined but not disturbed except for the temporary removal of the aeration pipette and watchglass. Notes are made on sediment appearance and unusual conditions. The number of amphipods that have emerged from the sediment, either floating on the water surface or lying on top of the sediment, is counted and recorded. Amphipods that have emerged are not removed, even if they are dead. These data are used to document the temporal pattern of emergence. Amphipods trapped by surface tension at the water surface are gently pushed down with a clean instrument (e.g., pipet, glass rod, beaker cover).

Termination--

The bioassay is terminated after 10 days of exposure. After daily observations are recorded, the contents of the bioassay beakers are sieved through a 0.5-mm screen. Material retained on the screen is placed in clean bioasssay water in a sorting tray. The number of live and dead amphipods is recorded. The sum of these numbers may not always equal 20 because of death and subsequent decomposition of amphipods. An amphipod is counted as alive if there is any sign of life (e.g., pleopod twitch observed under magnification, response to gentle prodding with a clean instrument).

Experimental Design

Logistics--

A typical sediment bioassay involves about 50 to 60 bioassay beakers. Collection and preparation of animals, sediment, and seawater requires at least four people for 2 days. Three or four people are required on the days experiments are initiated and terminated. One person can monitor the experiment in progress.

Controls--

Five replicates of the amphipod collection site control sediment are included in all bioassays. These beakers comprise a negative (clean) control that allows comparisons among experiments and among laboratories of the validity of the procedures used in individual investigations. At least 90 percent of the amphipods in the control replicates should survive. The design of field surveys may include a reference sediment involving five replicate samples from an area believed to be free from sediment contamination. This provides a site-specific basis for comparison of potentially toxic and nontoxic conditions. Experiments in which contaminants are added to sediment may require control replicates to determine effects of solvent addition.

A positive (contaminated) control is also required for all testing. This involves determining 96-h LC50 values for *R. abronius* exposed in clean, filtered seawater without sediment to reference toxicants (following standard bioassay procedures and under the same general test conditions as the sediment bioassays). Such data are necessary to determine the relative sensitivity of the animals (e.g., seasonal difference in sensitivity) for each test series to ensure comparability of the data. Two commonly used reference toxicants are reagent-grade $CdCl_2$ and NaPCP. Swartz et al. (1986) determined a 96-h LC50 of 1.61 mg/L for $CdCl_2$, while Cummins and Gangmark (in preparation) found that the 96-h LC50 for NaPCP ranged from 0.30 to 0.39 mg/L over a 2-yr period. Either reference toxicant may be used, but the acute lethality results must be reported along with the sediment bioassay results. Bioassays to establish an LC50 involve four or five logarithmic concentration series and a control. At least one treatment should give a partial response below

the LC50 and one above the LC50. Statistical procedures for the LC50 estimate are given in APHA (1985).

Response Criteria--

Emergence from sediment and survival are the two response criteria examined in all bioassays. Emergence data are used to monitor a behavioral response of the amphipods during the 10-day exposure. Survival after 10 days of exposure is the primary criterion of toxicity. Each of these response criteria must be monitored in a "blind" fashion; that is, the observer must have no knowledge of the treatment of the sediment in the beakers. This is accomplished through randomization of beaker numbers.

DATA REPORTING REQUIREMENTS

The following data should be reported by all laboratories performing this bioassay:

- Water quality measurements during testing (i.e., DO, temperature, salinity, pH).
- Daily emergence for each beaker and the 10-day mean and standard deviation for each treatment
- 10-day survival in each beaker and the mean and standard deviation for each treatment
- Interstitial salinity values of test sediments
- 96-h LC50 values with reference toxicants
- Any problems that may have influenced data quality.

BIVALVE LARVAE SEDIMENT BIOASSAY

USE AND LIMITATIONS

The bivalve larvae bioassay technique is described in Standard Methods (APHA 1985) and by ASTM (1985) as a rapid and reliable indicator of environmental quality. Pacific oysters (*Crassostrea gigas*) and blue mussels (*Mytilus edulis*) are recommended for testing. During the first 48 h of embryonic development, fertilized oyster and mussel eggs normally develop into free-swimming, fully shelled larvae (prodissoconch I). Failure of the eggs to survive or the proportion of larvae developing in an abnormal manner is used as an indicator of toxicity.

This sediment bioassay can be used to characterize the toxicity of marine sediments. It may be used alone as a screening tool in broad-scale sediment surveys, in combination with sediment chemistry and in situ biological indices, and in laboratory experiments addressing a variety of sediment and water quality manipulations.

The bivalve larvae bioassay can be used in sediments that have interstitial salinities less than 1 ppt, as the sediments are mixed and equilibrated with seawater prior to testing. However, because further testing is required to determine the validity of using this technique with such low salinity sediments, this bioassay is not recommended for sediments that have an interstitial salinity of less than 10 ppt. In addition, the following caveats apply:

- Bivalve larvae such as those of *C. gigas* normally are not associated with the types of sediments that generally are tested using this method. Hence, this bioassay is only an indicator of relative toxicity and is not usually of direct ecological significance.

- Spawning of *C. gigas* occurs naturally in the Puget Sound area in summer. The natural spawning period for *M. edulis* is late spring to early summer. Both of these bivalves can be induced to spawn at other times of the year, but may show decreased viability of gametes. Gamete viability may also vary depending on the brood stock used. Accordingly, a positive control is recommmended. This should comprise a 48-h EC50 measurement with a reference toxicant (either $CdCl_2$ or NaPCP) conducted in the absence of sediment.

- It is possible that abnormalities induced during testing may be underestimated due to poor recovery of living larvae from the sediments. Accordingly, it is recommended that

a few sediment samples from each set of bioassays conducted with this technique be examined to determine whether living larvae are present in the sediment. The results should be quantified and reported.

FIELD PROCEDURES

Collection

Both control and test sediment should be collected in solvent-cleaned glass jars having TFE-lined lids. Each jar should be filled completely with sediment to exclude air. A minimum sediment sample size of 20 g for each bioassay chamber is recommended for both kinds of sediment.

Processing

Both control and test sediment should be stored at 4° C in the dark. Holding time should not exceed 14 days.

LABORATORY PROCEDURES

The following procedures apply equally to larvae of both *C. gigas* and *M. edulis*, and are as described by Chapman and Morgan (1983) with the following changes incorporated:

- The salinity of test water is adjusted to 28 ppt
- Replication is increased from two to five to allow adequate statistical comparisons
- Larvae of *M. edulis* are included in the bioassay protocol
- Sediment resuspension in the bioassay containers is adequately accomplished by vigorous shaking for 10 sec; there is no need to rotate the containers for 3 h at 10 rpm
- Twenty grams of sediment is suspended in 1 L of seawater rather than 15 g in 750 mL
- Sediment holding time prior to testing is set at a maximum of 14 days
- pH is not adjusted before the bioassay starts and is only monitored
- A positive control is recommended
- Additional details provided by ASTM (1985) for conditioning and spawning adults are included.

Bioassay Species

For a given test or series of related tests, adult bivalves (brood stock) are obtained from the same source, either commercial rearing facilities (oysters) or a chemically uncontaminated area (mussels). Within 24 h of collection or purchase, adults should be transported to the test laboratory and placed into flowing seawater similar in character to that from which they were taken. Rough handling; extended periods of desiccation; and abrupt changes in temperature, salinity, or other water quality variables must be avoided as these may induce premature spawning or render the stock useless for later controlled spawning or both. Upon receipt, adults should be cleaned of fouling organisms and detritus and placed in flowing seawater for conditioning.

Adult bivalves are held at recommended conditioning temperatures to stimulate final maturation of the gametes. The desired conditioning temperature (20 $\pm 2^{o}$ C for oysters and 14 $\pm 2^{o}$ C for mussels) should be attained gradually at an increment not exceeding 2° C per day. Conditioning may extend from a few days to several weeks depending on the physiological and gametogenic status of the adults. The length of the conditioning period is determined empirically by periodic sacrifical examination and spawning of representative individuals. Adults should be spawned or discarded within 2-3 wk after attaining acceptable maturity because gamete quality will deteriorate rapidly with excessive conditioning.

Bioassay Sediment

The bivalve larvae bioassay is conducted with sediment controls in addition to seawater controls. Control sediment typically consists of material collected from an area documented to be free of chemical contamination and of proven nontoxicity to bivalve larvae.

Bioassay Seawater

Seawater used in the bioassay is maintained at a salinity of 28 ± 1 ppt and temperature of 20 $\pm 1^{o}$ C. The bioassay seawater must be uncontaminated and of acceptably low toxicity. The standard biological criterion of acceptability is that the larvae, spawned by adults in the dilution water, must not incur more than 10 percent abnormal development or 30 percent mortality during 48 h of exposure to the dilution water (ASTM 1985).

If necessary, salinity of the bioassay water is reduced by addition of deionized distilled water or raised by addition of clean oceanic water or reagent grade chemicals (ASTM Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians [E729]). Seawater is prepared within 2 days of use and is stored in clean, covered containers at the requisite temperature.

Facilities and Equipment

The bioassay chamber is a clean, acid-rinsed, 1-L glass bottle with a screw-top lid. Bioassays are conducted with the bottles in shallow water baths, incubators, or temperature-controlled rooms.

If adults are to be conditioned for spawning out-of-season, a continuous supply of temperature-controlled seawater is needed. Raw seawater can be used for holding and conditioning, but feeding the adults a natural or cultivated alga is necessary to deter starvation. The flow rates used for adult conditioning must be high enough to prevent water quality degradation and provide as much food as possible to the adults.

Tanks and trays are necessary for holding the adults, and a water bath, incubator, or temperature-controlled room is necessary during the bioassay. Adult holding and conditioning tanks should be cleaned at least weekly to prevent accumulation of organic matter and bacteria. Dead specimens should be removed immediately and the tanks cleaned. The tanks should be cleaned with detergent and rinsed with clean seawater, and if microbial growth is present, rinsed with 200 mg/L of hypochlorite and then seawater. With enriched waters and elevated conditioning temperatures, more frequent cleaning may be required. All new test chambers should be washed with detergent and rinsed in turn with water, pesticide-free acetone, water, acid, and then water.

Bioassay Procedure

The bioassay procedure can be summarized as follows. 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 control sediments for 48 h, during which the embryos normally will develop into prodissoconch I larvae. Toxicity is measured based on abnormal shell development and larval death.

The bivalves are spawned by rapidly raising the water temperature to 5-10° C above the conditioning temperature. Females are additionally stimulated to spawn by the addition of sperm from a sacrificed or naturally spawned male.

Spawning is conducted by placing the bivalves in individual, clean Pyrex dishes containing filtered, UV-treated seawater. Fertilization is accomplished within 1 h of spawning by combining eggs and sperm in a 1-L Nalgene beaker. The fertilized eggs are then washed through a 0.25-mm Nitex screen to remove excess gonadal material and suspended in 2 L of filtered, UV-treated seawater at incubating temperature. The embryos are kept suspended by frequent agitation using a perforated plunger and used in the bioassay within 2 h of fertilization. When microscopic examination of fertilized eggs reveals the formation of polar bodies, egg density is

determined from triplicate counts of the number of eggs in 1.0-mL samples of a 1:99 dilution of homogeneous egg suspension.

Sediment bioassays are conducted in clean (i.e., rinsed with dilute acid) 1-L glass bottles. Twenty grams (wet weight) of the appropriate sediment is added to each bottle and volume is brought up to 1 L with filtered, UV-treated seawater (28 ppt salinity) to make a final concentration in all containers of 20 g (wet weight) of sediment per liter of seawater. All test treatments are replicated five times. The sediment controls contain 20 g/L of clean sediment. In addition, a control series is prepared consisting of clean seawater without sediment.

The sediments are suspended by vigorous shaking for 10 sec, the embryos are added, and the suspended sediments are allowed to settle. No additional agitation is provided. The seawater control is treated similarly.

Within 2 h of fertilization, each container is inoculated with 20,000-40,000 developing embryos to give a concentration of about 20-40 per mL. The embryo concentration at 0 h should be confirmed by collecting replicate 10-mL samples from control cultures and preserving them in 5-percent formalin. The containers are covered and incubated for 48 h at 20 $\pm 1^{o}$ C under a 14-h light:10-h dark photoperiod. Test vessels are not aerated during the bioassay.

After 48 h, the bioassay can be terminated in one of two ways. In the first method, the contents of each container are carefully poured through a 38-um Nitex sieve without disturbing the settled sediment, thereby retaining and concentrating the surviving larvae (larvae caught in the sediments are almost always dead). The concentrated larvae are washed into a 100-mL graduated cylinder, quantitatively transferred to 8-mL screw-cap vials, and preserved in 5 percent buffered formalin. In the second termination method, the water and larvae overlying the settled sediment in each container are carefully poured into a clean 1-L beaker. This water is then stirred, and a 10-mL aliquot of the well-mixed sample is removed by pipet and placed in a 10-mL screw-cap vial. The contents of each vial is preserved in 5 percent buffered formalin.

Preserved samples (equal in volume to those containing 300-500 larvae in controls) are examined in Sedgewick-Rafter cells under 100X magnification. Normal and abnormal larvae are enumerated to determine percent survival and percent abnormality. Percent survival is determined as the number of larvae surviving in each test container relative to the seawater control. Larvae that fail to transform to the fully shelled, straight-hinged, 'D'-shaped prodissoconch I stage are considered abnormal.

Salinity and dissolved oxygen are initially adjusted in each container to 28 ppt and a minimum of 90 percent saturation, respectively. These variables and pH are measured in each container at the start and termination of the bioassay.

Controls

Five replicates of control sediment and five replicates of control seawater alone are included in all bioassays. These comprise negative (clean) controls that allow comparison among experiments and among laboratories of the validity of the procedures used in individual investigations. At least 70 percent of the larvae must survive the 48-h exposure with seawater alone, and of these at least 90 percent must show no abnormalities. The design of field surveys may include a reference sediment from an area believed to be free from chemical contamination. This provides a basis for comparison of potentially toxic and nontoxic conditions. Experiments in which contaminants are added to sediment may require control replicates to determine effects of solvent addition.

A positive (toxic) control is also required. This involves determining 48-h LC50 and EC50 values for bivalve larvae exposed to reference toxicants in clean, filtered, UV-treated seawater without sediment (following standard bioassay procedures and under the same general test conditions as the sediment bioassays). Such data are necessary to determine the relative sensitivity of the larvae. Two commonly used reference toxicants are reagent-grade $CdCl_2$ and NaPCP. Either reference toxicant may be used, but the results must be reported along with the sediment bioassay results. Bioassays to establish an LC50 or an EC50 involve four or five logarithmic concentration series and a control. At least one treatment should give a partial response below the LC50 and EC50 and one above the LC50 and EC50. Statistical procedures for the LC50 and EC50 estimates are given in APHA (1985) and ASTM (1985).

DATA REPORTING REQUIREMENTS

The following data should be reported by all laboratories performing this bioassay:

- Water quality measurements at the beginning and end of testing (e.g., dissolved oxygen, temperature, salinity, pH)
- Individual replicate and mean and standard deviation data for larval survival after 48 h
- Individual replicate and mean and standard deviation data for larval abnormalities after 48 h
- 48-h LC50 and EC50 values with reference toxicants
- Data on larval presence in the sediment
- Any problems that may have influenced data quality.

ANAPHASE ABERRATION SEDIMENT BIOASSAY

USE AND LIMITATIONS

This sediment bioassay is used to characterize the genotoxicity of marine sediments. It may be used alone as a screening tool in broad-scale sediment surveys, in combination with sediment chemistry and in situ biological indices, and in laboratory experiments addressing a variety of sediment and water quality manipulations.

This bioassay can be used with any type of sediment, regardless of the interstitial salinity or grain size characteristics. However, the following caveats apply:

- The bioassay depends on a chemical extraction procedure that is specific for neutral, nonionic organic compounds. Other classes of contaminants such as metals and highly acidic and basic organic compounds are not efficiently extracted. Thus, characterization of sediment toxicity is directed towards neutral compounds such as aromatic and chlorinated hydrocarbons.

- "Natural" genotoxicity may occur in marine sediments due to the decomposition of plant species containing genotoxic substances that evolved as a means of protecting plants from parasites and predators. Thus, positive genotoxic responses may be noted in areas generally regarded as pristine.

FIELD PROCEDURES

Collection

Sediment should be collected in solvent-rinsed glass jars having TFE-lined lids. Each jar should be filled completely to exclude air. A minimum sediment sample size of 100 g is recommended for each test.

Processing

Sediment samples should be stored frozen at -20° C within 8 h of collection. Holding time should not exceed 6 mo.

LABORATORY PROCEDURES

As previously mentioned, this technique depends on a chemical extraction procedure. Extraction procedures recommended are those presently used by the National Marine Fisheries Service, National Oceanic and Atmospheric Administration in Puget Sound (MacLeod et al. 1984).

The following procedure is synthesized from Kocan et al. (1982), Chapman et al. (1982), Kocan and Powell (1985), and Kocan et al. (1985). Accordingly, this procedure supersedes previous published methodologies.

Cell Cultures

Although any cell type can be used in this test, the rainbow trout gonad cell (RTG-2) is recommended because it has been used extensively for this purpose, is readily obtainable, and is easy to cultivate. It has numerous mitotic figures when growing exponentially and the mitotic cells are large in comparison to those of other species, thereby making it easy to count the damaged anaphase cells. This cell type is sensitive to a wide range of organic chemical compounds (Kocan et al. 1982) as well as complex mixtures of chemicals that may occur in marine sediments (Kocan and Powell 1985; Kocan et al. 1985). Other cells used for this purpose are the bluegill-sunfish line (BF-2), human foreskin fibroblasts, newt cells, and several plant cells (in vivo). Generally these cells can be obtained from any state or federal fish disease diagnostic laboratory, from investigators at both university and federal laboratories, or from the American Type Culture Collection (Rockville, MD).

Sediment Extraction

Test materials must consist of substances that are compatible with the growth of cells in culture. Generally this can be accomplished using organic extracts of environmental material such as marine sediments.

Extractions should be made using pesticide-grade reagents that have been tested for toxicity to the cells prior to their use for extraction. This initial toxicity testing can be done by evaporating a volume of the reagent equivalent to that which would be used for the actual extraction, and adding this to the cultures in varying amounts dissolved in the solvent (e.g., dimethyl sulfoxide, DMSO) to be used for cell exposure. If the maximum anticipated amount of the solvent blank to be used in the final test does not significantly affect the cell cultures, the extraction can proceed without concern about the possible toxic effect of the solvents. Once the extracts have been made, gravimetric determinations of their absolute organic content must be made so that comparable organic concentrations from each site can be used in cell cultures exposed to extracts from different locations. This is accomplished by first weighing the tube to be used for extract storage to the nearest 0.001 g, adding the extract in the solvent, evaporating the solvent with nitrogen, and reweighing the tubes. The difference

between the original tube weight and the final tube weight is the weight of the sample extract. The sample extract is then redissolved in spectral-grade DMSO so that each sample contains the same weight/volume ratio of extract (e.g., mg/mL). In this way it is possible to expose each set of cultures to exactly the same amount of organic extract, thereby making comparisons possible on an organic content basis. If relative toxicities from site to site are needed, one can extrapolate back to the original organic content of each sample and compare sites based on total amounts of organic compounds present. The following detailed procedures for the preparation of sediment extracts follow those of Chapman et al. (1982) and MacLeod et al. (1984).

Sediment samples are frozen and stored until just prior to extraction. Each sample is then thawed and rehomogenized by thorough stirring. An aliquot of approximately 20 g wet weight is transferred to a clean, tared beaker, dried to constant weight (80° C), desiccated, and reweighed to determine the percent water. A second aliquot (approximately 150 g wet weight) is transferred to a tared, solvent-cleaned, 315-mL stainless steel centrifuge bottle with a TFE-lined screw-cap, and weighed. The sample is then serially extracted with pesticide-grade solvents.

Methanol (50 mL) is added to each centrifuge bottle. The bottle is tightly capped, shaken vigorously for 2 min, and centrifuged at 2,000 rpm for 5 min. The clean solvent is decanted into a 1-L separatory funnel. The procedure is performed twice more and the methanol extracts are combined in the separatory funnel, which is then closed and covered with aluminum foil.

One hundred milliliters of a dichloromethane/methanol (2:1 v/v) solution is added to the centrifuge bottle, the cap is closed tightly, and the bottle is shaken vigorously for 2 min to ensure complete mixing. The bottle is then placed in a shaker table overnight (approximately 18 h), following which the sediment is settled by centrifugation at 2,000 rpm for 5 min and the solvent decanted into the separatory funnel with the methanol. A second 100-mL aliquot of the dichloromethane/methanol (2:1) solution is added, the bottle is shaken vigorously, and the bottle is then placed on the shaker table for 6 h. The sediments are again settled with centrifugation and the solvents are decanted.

The remaining sample is shaken vigorously for 2 min with approximately 30 mL of dichloromethane. It is then centrifuged and the solvent is decanted into the separatory funnel. Another 100 mL of dichloromethane is added to the bottle, the cap is secured, the bottle is shaken vigorously, and the bottle is placed on the shaker table overnight. The sediments are again settled with centrifugation and the solvent is decanted into the separatory funnel. A final 30-mL rinse of dichloromethane is added, the bottle is shaken vigorously, centrifuged, and decanted. The sediment is then discarded.

Approximately 500 mL of clean, distilled water is added to the combined solvents in the separatory funnel. The funnel is carefully swirled and inverted (with frequent venting) for 2 min. The liquid phases are allowed to separate and the dichloromethane (lower) layer is drained into a 500-mL separatory funnel. The aqueous layer is re-extracted twice with 20 mL of dichloromethane and the remainder is discarded. The dichloromethane fractions are combined in the 50-mL funnel, transferred, with rinsing, back to the 1-L funnel, and re-extracted with another 500 mL of distilled water. The dichloromethane is drained into the 500-mL funnel and the aqueous layer is extracted once more with 20 mL of dichloromethane. The latter solvent is added to the 500-mL funnel and the aqueous layer is discarded.

The dichloromethane is drained from the 500-mL separatory funnel through approximately 20 g of combusted and washed anhydrous sodium sulfate that is held in a 30-mL glass conical centrifuge tube with the tip cut off. The effluent from this mini-column is discharged into a 500-mL Kuderna-Danish flask with a 15-mL receiver. When empty, the 500-mL separatory funnel is rinsed with 20 mL of dichloromethane, which is drained through the sodium sulfate column into the flask. The column is washed a final time with 10 mL of dichloromethane and drained into the flask.

Boiling chips are added to the Kuderna-Danish flask and a 3-ball Snyder column is placed on top. The solvent volume is reduced to about 5 mL using a hot water bath. When cooled, the sides of the flask are rinsed into the receiver with dichloromethane. The receiver is removed and the contents are quantitatively transferred to a tared conical centrifuge tube with a ground glass stopper. The sample is taken almost to dryness using the hot water bath, and stored wrapped in aluminum foil in a desiccator with the stopper open slightly until a constant weight is achieved upon reweighing the tube. This weight is the amount of extractable organic material.

After weighing, the tube is closed and wrapped fully in aluminum foil, ready for anaphase aberration testing. Extracts are treated with 1 mL of spectrophotometric-grade DMSO for 24 h with frequent stirring on a vortex mixer. The DMSO is then removed to a glass vial and used as "stock" solution. Because all extracted material is not dissolved in the DMSO during testing, the centrifuge tubes are dried and reweighed to determine the exact amount used in testing (fraction soluble). Both stock and extract solutions are stored in the dark under nitrogen until applied to the cell cultures.

Culture Conditions

RTG-2 cells as well as most fish cell lines grow in a variety of commercially available culture media. The Leibovitz L-15 medium was found to be most consistent in terms of ease of preparation, use without special buffers or carbon dioxide incubators, and long-term storage capability. This medium can be obtained from any scientific supply house that carries cell/tissue culture materials. The medium comes as a dry powder that is added to distilled water, autoclaved or filter-sterilized, and stored in

a refrigerator at 4° C until it is used. Full instructions are available with the medium when it is purchased. Generally, heat-deactivated fetal calf serum is added to any culture medium at 10 percent concentration to ensure that the proper growth factors are present. This serum can also be purchased in 100-mL to 1-L lots already sterilized from the scientific suppliers. Before the cells are placed in the medium, pH should be adjusted to 7.1-7.3 using either sodium bicarbonate or HCl. Some laboratories use HEPES buffer (either acid or base in a commercially available product) in place of the bicarbonate or HCl. This culture system differs from that originally described by Kocan et al. (1982) only because it has been modified to simplify the laboratory procedures.

Bioassay Procedure

Cells are grown and tested at 18° C on standard, clean microscope slides or on 1x5 cm coverslips in Leighton tubes, depending on the amount of test material available. The cells are placed into the culture system 1 day prior to the actual exposure to ensure that they have had a chance to attach to the glass substrate and begin growing. On the following day (18-24 h later), the culture medium is removed and the test material is added. This should consist of normal L-15 medium dissolved in DMSO, to which the organic extract has been added. The DMSO should be from a pretested lot to ensure that it is nontoxic to the system and should not exceed 0.5 percent (v/v) of the culture medium (e.g., 5 uL/mL). This can be reduced to 0.1 percent DMSO if toxicity is a problem or if minimal DMSO is required for conservation of extract. Exposure time should be 48 h from the time of addition of the treated medium until fixation. Damaged cells can be observed for longer periods even after the toxic substance has been removed, but the maximum response does not increase beyond 48 h.

An initial screening test must be conducted to determine the actual extract dilutions to be used for this bioassay. Ideally, dilutions tested for anaphase aberrations comprise both the highest concentration of extract (mg/L) that permits continued cell proliferation (i.e., nontoxic) and a second concentration one dilution lower. This method ensures that a sufficient number of mitotic figures are present to score for chromosome damage. Based on previous experience in Puget Sound, the following six extract dilutions should be prepared: 50, 25, 10, 5, 2 and 1 ug/mL. Cells are first exposed to these concentrations for each sediment extract tested, and then the concentrations that inhibit mitosis are determined. Although identical extract dilutions may not be used to test each sediment sample, all results are normalized to organic content which has been previously determined.

To determine mitotic effects and anaphase aberrations, the slides or coverslips containing the cells are removed from the culture medium and fixed in methanol:acetic acid (3:1). The methanol is absolute and the acetic acid is glacial (undiluted). Following 15-60 min in the fixative (no adverse effects occur if they are left longer) the slides are air dried,

then placed into 3 percent Gurr's R66 Geimsa stain for 15-30 min. This stain is made up in Sorensen's buffer (pH 6.8). Optimum staining is determined empirically by examining the slides with a microscope at various intervals after placing them in the stain. Problems usually occur because of too little staining time rather than excessive staining. If excessive staining does occur, the cells can be destained in Sorensen's buffer. The staining system selectively stains the condensed chromosomes undergoing mitosis and has very little effect on the cytoplasm of the cell, which allows good resolution of the small fragments of chromosome that are not associated with the main chromosome bundles.

Once the cells have been stained, they are mounted on microscope slides (if on coverslips) or are covered with coverslips (if grown on slides) to facilitate microscopic examination and scoring. Slide identification labels are covered with a piece of tape to prevent observer bias while scoring. Three replicate slides are made of each exposure concentration with two concentrations for each sediment extract. Each slide is then examined at 500X to 1,000X until a minimum of 100 anaphase cells is observed and scored. In this way, there will be three replicates per dose with 100 anaphase cells per replicate.

The numbers and percents of normal and abnormal anaphases are recorded. Cells are scored as abnormal if they contain any of the previously described chromosomal lesions reported for this test (Nichols et al. 1977; Kocan et al. 1982; Chapman et al. 1982a; Kocan and Powell 1985; Kocan et al. 1985).

Controls

Controls consist of 1) untreated cultures used as negative controls to ensure that the culture conditions (e.g., medium, serum) are not toxic to the cells, 2) a solvent blank to ensure that the residue from the solvents used during the extraction procedure is not cytotoxic or genotoxic (generally done prior to actual testing and includes the DMSO that will be used in the final solution), and 3) a positive control consisting of cultures exposed to several concentrations of a known genotoxic agent to indicate that the test system is functioning properly and does indeed respond to genotoxic substances. One possible positive control is a 0.25-ug/mL concentration of benzo(a)pyrene. This level of exposure should result in an anaphase aberration frequency of 50-65 percent (Chapman et al. 1982a).

DATA REPORTING REQUIREMENTS

The following data should be reported by all laboratories performing this bioassay:

- Initial screening data for the determination of extract concentrations

- Individual replicate and mean and standard deviation data for numbers of normal and abnormal anaphases observed
- Types of anaphase aberrations observed
- Frequency of anaphase aberrations observed with the positive control
- Any problems that may have influenced data quality.

MICROTOX SEDIMENT BIOASSAY (ORGANIC EXTRACT)

USE AND LIMITATIONS

The Microtox bioassay is a rapid, sensitive method of toxicity testing based on light emission by the luminescent bacterium (*Photobacterium phosphoreum*) in the presence and absence of aqueous toxicants. The emitted light is a product of the bacterial electron transport system and thus directly reflects the metabolic state of the cell. Accordingly, decreased luminescence following exposure to chemical contaminants provides a quantitative measure of toxicity. The assay was developed for use in freshwater habitats to assess the toxicity of waterborne pollutants (Bulich et al. 1981) and has been adapted for use in the marine environment to assess toxicity of organic sediment extracts (Schiewe et al. 1985).

This sediment bioassay is used to characterize the toxicity of marine sediments. It may be used alone as a screening tool in broad-scale sediment surveys, in combination with sediment chemistry and in situ biological indices, and in laboratory experiments addressing a variety of sediment and water quality manipulations.

The Microtox bioassay can be used with any type of sediment, regardless of interstitial salinity or grain size characteristics. However, the following caveats apply:

- The bioassay depends on a chemical extraction procedure that is specific for neutral, nonionic organic compounds. Other classes of contaminants such as metals and highly acidic and basic organic compounds are not efficiently extracted. Thus, characterization of sediment toxicity is directed towards neutral compounds such as aromatic and chlorinated hydrocarbons.

- Naturally occurring toxic substances may be present in and extracted from marine sediments. Hence, relatively high toxicity occasionally may be noted in areas generally regarded as free from chemical contamination.

FIELD PROCEDURES

Collection

Sediment should be collected in solvent-rinsed glass jars having TFE-lined lids. Each jar should be filled completely to exclude air. A minimum sediment sample size of 500 g is recommended for each test.

Processing

Sediment samples should be stored frozen at -20° C within 8 h of collection. Holding time should not exceed 6 mo.

LABORATORY PROCEDURES

Facilities and Equipment

The bioassay is performed using the Beckman Model 2055 Microtox Toxicity Analyzer System, a temperature-regulated photometer equipped with a photomultiplier. Freeze-dried bacteria, reconstitution solution (i.e., organic-free distilled water), diluent, and other necessary materials can be purchased from Microbics Corporation, Carlsbad, California. The test procedure is conceptually quite straightforward. However, the methodology requires careful attention to detail and requires 1-2 wk to become technically proficient.

Sediment Extraction

As previously mentioned, this technique depends on a chemical extraction procedure. Extraction procedures recommended are those presently used by the National Marine Fisheries Service, National Oceanic and Atmospheric Administration in Puget Sound (MacLeod et al. 1984).

- Thaw sediment, decant and discard excess water. Homogenize sediment with stainless steel spoon. Discard large pebbles, shells, seaweed, wood, crabs, etc.
- Weigh out 10 ±0.5 g sediment to the nearest 0.01 g and place in a dichloromethane-rinsed centrifuge bottle
- Set aside approximately 10 g of the homogenized sediment for the determination of dry weight
- Centrifuge sediment sample for 5 min at 1,000 x g and discard water
- Add 100 mL spectral-grade dichloromethane and 50 g sodium sulfate to sediment sample

- Manually shake bottle until contents are loose and free-flowing and then roll 16 h (overnight) on a tumbler
- Centrifuge sediment 5 min at 1,000 x g and save the dichloromethane extract
- Add another 100 mL dichloromethane to sediment and tumble for 6 h (during day)
- Centrifuge for 5 min at 1,000 x g and save the dichloromethane extract
- Repeat extraction a third time with 100 mL dichloromethane for 16 h (overnight) and collect extract by centrifugation
- Combine the three 100-mL portions of the dichloromethane extract
- Add three or four boiling chips to flask containing the dichloromethane extract and attach to Synder column
- Concentrate extract to 10-15 mL in a 60° C water bath and then transfer it to a concentrator tube
- Wash down the flask two times with 3-4 mL dichloromethane and add washings to the tube
- Add a boiling chip to the tube and, using a tube heater, concentrate the extract to greater than 0.9 but less than 1.0 mL
- Adjust volume to 1.0 mL with dichloromethane (at this point 0.1 mL may be removed for GC/MS analyses)
- Add 3 mL of hexane to the remaining 0.9 mL of extract and concentrate to 2 mL
- Remove 100 uL of extract and add to a concentrator tube containing 3 mL ethanol
- Place tube in heater block and concentrate to 2 mL
- Adjust volume to 3.0 mL with ethanol (the standard sediment extract used in Microtox testing)
- Extraction blanks are prepared using an identical procedure but without the sediment.

Bioassay Procedure

The organic extract approach uses the basic Microtox method described in the Microtox Operating Manual (Beckman Instruments 1982) and by Bulich et al. (1981).

- Reconstitute lyophilized bacteria with 1-mL double-distilled, charcoal-filtered water in a Microtox cuvette and place in 4° C holding well. Bacterial suspensions are used within 8 h of reconstitution.

- Prepare three or more widely spaced primary dilutions (e.g., 5.0, 0.5, 0.05 percent extract [v/v]) in double-distilled, charcoal-filtered water.

- Adjust concentration of each primary dilution to 2 percent NaCl by adding 0.1 mL 22 percent NaCl per mL of diluted extract.

- Use these diluted extracts in a range-finding assay to determine an appropriate primary dilution for the definitive assay described below. The primary dilution should cause a 65-90 percent decrease in bioluminescence in 15 min. Methods for the range-finding assay are the same as those for the definitive assay except usually only three concentrations are tested without replication.

- For the definitive assay, two-fold serial dilutions (i.e., 5.0, 2.5, 1.25, and 0.625 percent) of extract are prepared in 2 percent NaCl. A 2 percent NaCl blank is also prepared for testing to measure spontaneous decay of light production which occurs naturally independent of treatment.

- In each of 10 test cuvettes, a 10 uL aliquot of bacterial suspension is added to 500 uL of diluent and incubated for 15 min in the incubation wells. This assures temperature equilibration and stability of bioluminescence.

- After 15 min, initial levels of light emission are measured in each of the 10 test cuvettes.

- At 30-sec intervals, 500 mL aliquots of each concentration of extract are added to two of the cuvettes (i.e., two replicates each of the four extract dilutions and the saline blank). Timing is critical because bioluminescence gradually decreases over time.

- Exactly 5 min after addition of the sediment extract, light emission is measured at 30-sec intervals and in the same sequence used for extract additions in the preceding step. Light emission is measured again at 15 min and 30 min.

- Immediately after testing each sediment extract, an ethanol-only control is assayed using the same primary dilution sequence used in the sediment extract test. The ethanol-only data are used to adjust the sediment extract data for the contribution of the solvent vehicle.

- Estimates of the 15-min EC50s (i.e., the concentration of extract causing a 50 percent reduction in bioluminescence) are obtained using linear regression analyses (see Microtox Operating Manual). Briefly, the percent inhibition of light emitted at each test concentration and time point is converted to a gamma value which is defined as the ratio of light lost to light remaining. Gamma values are normalized for natural decline in light production measured over time as described by Bulich et al. (1981) and further adjusted for the contribution of the ethanol vehicle. The natural log of gamma is regressed on the natural log of extract concentration and the EC50 is calculated from the regression equation. A statistical procedure based on Fieller's theorem (Finney 1964) is used to calculate a 95-percent confidence interval.

Controls

- Ethanol, sodium lauryl sulfate, or other suitable compound should be used to assess daily bioassay performance and to determine differences in response among lots of bacteria.

- Bioassay repeatability is evaluated by duplicate testing of 10 percent of the sediment extracts.

DATA REPORTING REQUIREMENTS

The following data should be reported by all laboratories performing this bioassay:

- Range-finding assay results

- Raw light emission data for each test series

- 15-min EC50 data and 95-percent confidence intervals for each test series and for controls

- Any problems that may have influenced data quality.

MICROTOX BIOASSAY (SALINE EXTRACT)

USE AND LIMITATIONS

The use of saline extracts of sediment for the Microtox bioassay has been described by Williams et al. (in press). This technique is not presently recommended for widespread use in Puget Sound as it requires further research to evaluate test variables and effects of sediment storage and extraction techniques on the precision of the results. However, the techniques are well developed and are provided herein for ease of use in possible future research efforts.

The organic and saline extract approaches both use the basic Microtox method described in the Microtox Operating Manual (Beckman Instruments 1982) and by Bulich et al. (1981). The major difference is in the preparation of test samples. Each procedure is specific with regard to the classes of contaminants that are tested for toxicity and, in general, the results for each approach can be viewed as complementary.

A limitation of the saline extract Microtox bioassay procedure is that only the water-soluble fraction of sediment-adsorbed trace metals and organic pollutants are extracted from the sediments. Thus, contaminants with extremely low water solubilities (PCBs) will tend to be partitioned almost exclusively onto sediment particles and are unlikely to occur in high concentration in the saline extract used in toxicity testing. Another limitation is the need to establish a correction factor for changes in bacterial luminescence caused by variation among samples in sediment pore-water salinity. Although Williams et al. (in press) showed that salinity-induced changes in luminescence were negligible for sediments taken from Commencement Bay, other estuarine sediments may have a greater range of pore-water salinities and may require a salinity correction factor. Finally, the assay requires further research to demonstrate precision of the extraction procedure and stability of sediment toxicity during periods of prolonged sediment storage (i.e., 2 wk).

FIELD PROCEDURES

Collection

Sediment should be collected in solvent-rinsed glass jars having TFE-lined lids. Each jar should be filled completely to exclude air. A minimum sediment sample size of 200 g is recommended for each test.

Processing

Sediment samples should be stored at 4° C in the dark. Holding time should not exceed 14 days.

LABORATORY PROCEDURES

With slight modification, sample processing follows procedures described by Williams et al. (in press). Precleaned glass test tubes are filled with a minimum of 60 g of sediment, sparged with high purity nitrogen gas, tightly sealed with TFE-lined caps, stored in the dark at 4° C, and assayed within 14 days following collection.

Preparation of Sediment Extract

- Remove 30 g of sediment from each test tube with a stainless steel spatula, place in 30-mL glass containers equipped with a fritted glass cap, and add 10.0 mL of Mirotox diluent (2.0 percent NaCl w/v in double-distilled, organic-free water).

- Wash the sediment-diluent slurry for 24 h in the dark at 4° C by gentle agitation (100 rpm) on a rotary shaker table.

- Transfer the sediment slurry to 30-mL Corex tubes and centrifuge for 15 min at 9,000 rpm (9,770 x g) in a refrigerated (4° C) centrifuge.

- Draw the supernatant off by pipette, place it in a clean test tube, cool on ice and use immediately in preparation of serial dilutions for the Microtox bioassay.

Bioassay Procedure

- Rehydrate a vial of freeze-dried bacteria with 1.0 mL of reconstitution solution, cover with parafilm, store in a 4° C well on the Microtox analyzer, and use within 5 h of rehydration.

- Prepare 100, 50, 25, 12.5 and 0 percent serial dilutions of the sediment supernatant in Microtox diluent. The 0 percent dilution is a reagent blank needed to measure spontaneous decay in bacterial luminescence independent of any treatment.

- In each of 10 test cuvettes, add 10 uL of the rehydrated bacterial suspension to 350 uL of diluent and incubate for 15 min in one of the 15° C wells on the analyzer. This assures temperature equilibration of the bacterial suspension and stability of luminescence.

- After 15 min, measure initial luminescence in each of the 10 test cuvettes.

- At 30-sec intervals, add 500 uL aliquots of each supernatant dilution to two of the cuvettes (e.g., two replicates each of the four test dilutions and the saline blank). Timing is critical because toxicant-induced decrease in luminescence begins as soon as the sediment supernatant is added to the bacterial suspension.

- Exactly 15 min after addition of the sediment supernatants, measure luminescence at 30-sec intervals and in the same sequence used for supernatant additions in the preceding step.

- Calculate percent decrease in luminescence relative to the reagent blank using the formula:

$$\text{Percent Decrease} = [(RI_o - I_t)/(RI_o)] \times 100$$

where:

I_o = initial luminescence
I_t = luminescence at the end of 15 min
R = blank ratio.

The blank ratio is calculated by:

$$R = B_t/B_o$$

where:

B_o = initial luminescence of the reagent blank
B_t = luminescence of the reagent blank after 15 min.

Controls

- Clean reference sediments used as negative controls.

- Construction of a calibration curve to determine salinity induced changes in bacterial luminescence.

- Use of sodium arsenate as a reference toxicant to assess day-to-day performance of the bioassay and to determine differences in toxic response among lot numbers of bacteria.

- Verification of a dose-response relationship between bacterial luminescence and sediment extract concentration.

DATA REPORTING REQUIREMENTS

The following data should be reported by all laboratories performing this bioassay:

- Percent decrease in luminescence for each concentration of supernatant (e.g., saline sediment extract) tested.
- Determination of a significant dose-response relationship by least-squares regression of percent decrease in luminescence on the logarithm of sample dilution.
- Determination of EC50 values and 95-percent confidence limits for the reference toxicant.

OTHER PROMISING TECHNIQUES

This section describes promising sediment bioassay techniques that may be generally applicable in Puget Sound following further detailed testing and validation of the methods. The techniques are listed in terms of relative priority:

1. The echinoderm sperm bioassay has been used in sediment bioassays using elutriates but may be affected by the physical presence of particles, masking any chemical toxicity (Ross et al. 1984; Malins et al. 1985; Dinnel and Stober 1985). If this test can be successfully adapted to sediment bioassay testing, it should prove useful. Echinoderms such as sea urchins and sand dollars may be usable interchangeably, allowing testing to occur over most of the year. Draft protocols have been developed by the U.S. EPA (Narragansett Laboratories) for conducting effluent toxicity tests with the sea urchin *Arbacia punctulata* (Nacci et al. in review). These protocols may be applicable in large measure to Puget Sound sediment testing. In addition to the echinoderm sperm bioassay, the echinoderm embryo bioassay described by Dinnel and Stober (1985) may also be applicable to sediment testing (Ross et al. 1984), allowing a wider range of life-cycle testing for this group of organisms.

2. Extension of the bivalve larvae sediment bioassay technique described earlier to bivalve species other than the Pacific oyster and blue mussel should be possible. ASTM (1985) has standardized procedures for only four species: Pacific oyster (*Crassostrea gigas*), blue mussel (*Mytilus edulis*), Eastern oyster (*Crassostrea virginica*), and quahog (*Mercenaria mercenaria*). APHA (1985) only discusses larvae tests with oysters. However, Puget Sound species such as the Olympia oyster (*Ostrea lurida*), *Macoma balthica*, and geoduck (*Panopea generosa*) may be useful alternative species for use in the bivalve larvae sediment bioassay.

3. Three techniques other than the echinoderm sperm test that were listed in Table 3 but not recommended for widespread use in Puget Sound are: 1) the oligochaete, *Monopylephorus cuticulatus*, respiration rate test; 2) the surf smelt, *Hypomesus pretiosus pretious*, partial life-cycle test; and, 3) the copepod, *Tigriopus californicus*, partial life-cycle test. These tests are all sensitive methods, but are not immediately applicable. The oligochaete respiration test and the copepod

partial life-cycle test are highly promising tests that, if further developed, may be suitable for inclusion in future updates of this document. The surf smelt egg to larvae test may be useful if natural variability can be overcome. In any case, some type of fish egg development test (e.g., herring or cod) would be extremely valuable for future use in Puget Sound, even though it is recognized that such species cannot be tested year-round.

4. Life-cycle bioassays with nematodes as mentioned in Table 2, although not used to date in Puget Sound, may provide a rapid and effective means of determining chronic toxicity of sediments.

5. The U.S. EPA (Narragansett Laboratories) is presently developing a solid phase sediment toxicity test using the infaunal amphipod Ampelisca abdita (Gentile et al. 1985). This species apparently is similar to Rhepoxynius abronius with respect to toxicant sensitivity. However, it is found in fine sediments, whereas R. abronius is not (Scott, K.J., 31 October 1985, personal communication). Ampelisca spp. also occur along the west coast of North America, and this animal may be a useful species for testing those fine sediments that may have some measure of physical effect on R. abronius.

6. The U.S. EPA (Narragansett Laboratories) is presently finalizing testing protocols for complex effluents in marine waters. Four such tests have been developed: 1) 7-day survival/growth/reproduction with the mysid, Mysidopsis bahia; 2) larval fish growth/survival with the sheepshead minnow, Cyprinodon variegatus; 3) sea urchin (Arbacia punculata) sperm cell tests; and 4) sexual reproduction tests with the alga Champia parvula. The sea urchin sperm cell test is already noted in this document as a promising technique. The other three tests may be useful methods to test the toxicity of sediment elutriates.

7. The Federal Register (U.S. EPA 1985) recently introduced formal test guidelines for several types of bioassays under the Toxic Substances Control Act. Two of these tests are not listed above: algae and penaeid shrimp acute tests. These may be useful methods to test the toxicity of sediment elutriates.

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FISH PATHOLOGY

FINAL REPORT
TC-3991-04

Puget Sound Estuary Program

RECOMMENDED PROTOCOLS FOR MICROBIOLOGICAL STUDIES IN PUGET SOUND

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INTRODUCTION

This chapter presents recommendations for measuring microorganisms in Puget Sound. The recommendations are based on the results of a workshop and on written reviews by representatives from most organizations that fund or conduct microbiological studies in the Sound (Table 1). The purpose of developing these recommendations is to encourage all Puget Sound investigators conducting monitoring programs, baseline surveys, and intensive investigations to use standardized methods whenever possible. If this goal is achieved, most data collected in Puget Sound should be directly comparable and thereby capable of being integrated into a sound-wide database. Such a database is necessary for developing and maintaining a comprehensive water quality management program for Puget Sound.

The initial section of this chapter describes those microorganisms currently being measured in Puget Sound. In subsequent sections recommended microorganisms for future studies are identified, and special considerations for sampling water, sediment, and tissue are discussed. Finally, the uses and limitations of the recommended microorganisms are discussed, and laboratory and quality assurance/quality control (QA/QC) procedures are recommended.

It is recognized that departures from the general recommendations made herein may be necessary to meet the special requirements of individual projects. If such departures are made, however, the funding agency or investigator should be aware that the resulting data may not be comparable with most other data of that kind. In some instances, data collected using different methods may be compared if the methods are intercalibrated adequately.

TABLE 1. CONTRIBUTORS TO THE MICROBIOLOGY PROTOCOLS

Name	Organization
Carlos Abeyta	U.S. Food and Drug Adminstration
John Armstrong	U.S. Environmental Protection Agency
Scott Becker	Tetra Tech, Inc.
Norma Christopherson	Washington Department of Social and Health Services
Alfred Dufour	U.S. Environmental Protection Agency
Gary Fraser	Washington Department of Social and Health Services
Mike Glass	Washington Department of Social and Health Services
Peggy Hammer	U.S. Environmental Protection Agency
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Larry Kirchner	Seattle-King County Health Department
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Steve Martin	U.S. Army Corps of Engineers
Jack Matches	University of Washington
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Peter Nix	E.V.S. Consultants, Inc.
Mike Schiewe	National Oceanic and Atmospheric Administration
Jay Vasconcelos	U.S. Environmental Protection Agency
Marleen Wekell	U.S. Food and Drug Administration

MICROBIOLOGICAL MEASUREMENTS CURRENTLY MADE IN PUGET SOUND

A variety of agencies sample for bacteria in the water, sediment, or biota of Puget Sound. These agencies and the various bacterial indicators (i.e., of pathogens) and/or primary pathogens they evaluate are listed in Table 2. Measurements of bacterial indicators that are currently used include:

- Total counts of coliform bacteria (i.e., total coliform bacteria)
- Counts of fecal coliform bacteria (i.e., fecal coliform bacteria)
- Counts of *Escherichia coli* as a fraction of fecal coliform bacteria (i.e., fecal coliform bacteria/*E. coli*)
- Counts of enterococci, a subset of fecal streptococci (i.e., enterococci)
- Counts of *Clostridium perfringens* (i.e., *C. perfringens*)
- Counts of heterotrophic bacteria by standard plate count methods (i.e., standard plate counts).

TABLE 2. CURRENT BACTERIOLOGICAL MEASUREMENTS IN PUGET SOUND[a]

Agency	Routine Monitoring[b] Water	Routine Monitoring[b] Tissue	Special Projects
U.S. FDA (Seattle)	Total coliform bacteria (I) Fecal coliform bacteria (I) Fecal streptococci (I,NR)	Total coliform bacteria (I) Fecal coliform bacteria (I)	*Vibrio* spp. (P) *C. perfringens* (P) *Aeromonas hydrophila* (I,P) *Yersinia* spp. (P)
U.S. EPA (Region 10)	-	-	Occasional (most work is in fresh water)[c]
Seattle-King Co. Health Dept.	-	Fecal coliform bacteria (I)(NR)	-
Wash. Dept. of Ecology	Total coliform bacteria (I) Fecal coliform bacteria (I)	-	-
Metro	Fecal coliform bacteria (I)	Fecal coliform bacteria (I)	*C. perfringens* (I,P) *E. coli* (I) *Vibrio parahaemolyticus* (P) Enterococci (I)
Wash. Dept. of Social and Health Services	Fecal coliform bacteria (I)	Fecal coliform bacteria (I) Standard plate counts (I)	-

[a] I = indicator of primary pathogens or specific pollution sources, P = primary pathogen, NR = not routine.

[b] No agency in Puget Sound conducts routine monitoring of bacteria in sediments.

[c] Marine studies have included the fate and survival of fecal coliform bacteria and heterotrophic bacteria in relation to dredging operations.

RECOMMENDATIONS FOR FUTURE STUDIES

BACTERIAL INDICATORS

Reliance on a single bacterial indicator or a suite of indicators may not be desirable as a general rule because the objectives of different studies may vary substantially. For example, requirements for water pollution monitoring differ from those for evaluating the acceptability of shellfish for human consumption. In general, however, the following bacterial indicators should be considered for use in most surveys of bacterial indicators in Puget Sound:

- Fecal coliform bacteria
- Fecal coliform bacteria/*E. coli*
- Enterococci
- *C. perfringens*.

These indicators might be used individually or in various combinations depending upon the specific objectives of each study. The best uses and the limitations of each indicator are discussed later in this chapter.

PRIMARY PATHOGENS

In some cases, disease outbreaks may require the direct investigation and identification of primary pathogens because use of an indicator is inappropriate. For example, outbreaks of acute gastroenteritis may show no correspondence between the presence of a specific indicator, such as fecal coliform bacteria and the primary pathogen, such as *Yersinia enterocolitica* (Munger et al. 1980). Direct identification of primary pathogens may also be appropriate during reconnaissance surveys in areas lacking historical data. The choice of pathogen should be determined by the nature of the disease and by seasonal considerations. For example, *Vibrio* bacteria generally are found in the warmer months, whereas *Yersinia* bacteria are more prevalent during colder periods of the year.

SPECIAL SAMPLING CONSIDERATIONS

Choice of an optimum matrix for sampling (e.g., water column, sediment, interstitial water, or tissue) will depend upon the objectives of each study. For example, public health impacts are most likely through ingestion of contaminated shellfish or contact with contaminated water. Shellfish tissues or recreational waters may therefore be the materials most appropriate for analysis in studies focusing on health risks. The identification of present point sources of pollution can best be determined by analysis of water samples, whereas long-term pollution trends may best be described by sediment analysis.

WATER COLUMN

Water sampling can result in highly variable data because bacteria are not uniformly distributed throughout the water column (Gameson 1983) and sample volumes generally are limited to 50-100 mL. One major cause of spatial heterogeneity is the tendency for bacterial cells to concentrate in a thin microlayer on the surface of the water. Because bacterial abundances in the microlayer may exceed abundances in underlying surface water by several orders of magnitude (Hardy 1982), it is recommended that the microlayer and underlying water be sampled separately. However, sampling of the microlayer requires specialized techniques that have yet to be standardized. Also, collection and analysis of samples from both the microlayer and underlying water at each station may be too expensive for many routine monitoring programs. Thus, if separate samples cannot be collected within the constraints of a particular program, it is recommended that the microlayer be included in the sample by using the traditional "scoop" method of surface water sampling (U.S. EPA 1978). This method involves plunging an open bottle straight down to a depth of 15-30 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. It is recommended that samples be collected using a wide-mouth (12-15 cm) bottle to facilitate inclusion of the microlayer.

SEDIMENTS

Sediments are known to be heterogeneous with respect to types and numbers of bacteria. In addition, the bacteriological composition of sediments may have little relationship to public health impacts. For these reasons, routine monitoring of sediments in Puget Sound presently is not undertaken by any organization (Table 1), and is not recommended except under special circumstances.

One special application of sediment monitoring is analysis for *C. perfringens* to trace the distribution of sewage. Because spores of this bacterium

are associated with fecal pollution and survive over long periods, they offer the advantage of providing a cumulative record of sewage influence suitable for long-term monitoring surveys. However, the fact that the spores are persistent in the environment, and thus accumulate, renders analysis for C. perfringens in sediments inappropriate as a basis for providing regulatory guidelines.

TISSUE

Sampling and analysis of shellfish tissue present fewer problems than sampling and analysis of water and sediment (see APHA 1985a). Shellfish sampling is very important because the consumption of shellfish as food, sometimes in the raw state, may present a serious public health hazard.

Shellfish offer several advantages for sampling: they concentrate bacteria, can be sampled relatively easily, and reflect pollution levels over relatively long periods in both sediment and water. In Puget Sound it is recommended that one or several shellfish species of recreational or commercial importance be sampled routinely at each major harvesting area. The use of a small number (preferably one) of species as standards will reduce the variation among stations and sampling periods that results from interspecific differences in the propensity to concentrate bacteria. Because the whole organism is eaten, the whole body should be prepared for analysis.

USES AND LIMITATIONS OF RECOMMENDED BACTERIAL INDICATORS

Recommended bacterial indicators for monitoring in Puget Sound are presented in Table 3 for each kind of sample matrix.

FECAL COLIFORM BACTERIA AND FECAL COLIFORM BACTERIA/*E. COLI*

The density of fecal coliform bacteria has commonly been used as an indicator of fecal pollution and as an indicator of the presence of pathogens. The widespread use of fecal coliform bacteria as an indicator has the advantage of providing a basis for comparisons with historical data. However, there are several distinct, and often substantial, limitations to using fecal coliform bacteria for these purposes. In addtion, the densities of coliform bacteria may not accurately reflect public health risks (Hanes and Fragala 1967). Recent research has indicated that, although many species of non-pathogenic enteric bacteria (e.g., coliform bacteria) are viable in aquatic systems, they are not totally recoverable using conventional techniques (Roszak et al. 1984; Xu et al. 1982). Normal culturing techniques may seriously underestimate the concentrations of these organisms in the environment. Because the degree of recoverability may also depend on variable environmental factors (e.g., nutrient concentrations), it may be difficult to develop general bacteriological standards that could apply to all areas.

Cabelli et al. (1983) concluded that fecal coliform bacteria were inferior to enterococci as indicators of the presence of pathogens in marine recreational waters with respect to evaluating public health risks. Survival times for coliform bacteria are substantially less than for many pathogens (Borrego et al. 1983), complicating efforts to correlate counts of fecal coliform bacteria with the densities of pathogens at any specific time. Another problem associated with the use of fecal coliform bacteria as indicators is the fact that they are not specific to mammalian fecal pollution. For example, the fecal coliform bacterium *Klebsiella* is common in pulp mill effluents.

For all of the above reasons, data on fecal coliform bacteria cannot in themselves be considered adequate for a thorough assessment of public health risks. Fecal coliform bacteria continue to be used as an indicator because other indicators also have deficiencies, and because measurements of fecal coliform bacteria provide a basis for comparisons with historical data. The lack of specificity of fecal coliform bacteria to mammalian fecal pollution prompted the development of a membrane filtration method for enumerating *E. coli* (Dufour et al. 1981). Unlike fecal coliform bacteria as a group, *E. coli* are specific to mammalian fecal pollution. The enumeration method incorporates a technique for distinguishing *E. coli* from other fecal coliform bacteria. The result is notated as fecal coliform bacteria/ *E. coli*. However, with respect specifically to recreational water quality criteria,

TABLE 3. RECOMMENDED BACTERIAL INDICATORS FOR MONITORING IN PUGET SOUND

Objective	Matrix		
	Water[a]	Sediment	Tissue
Recreational use evaluation (e.g., swimming)	Fecal coliform bacteria Enterococci	-	-
Pollution monitoring and/or water quality surveys	Fecal coliform bacteria *E. coli* *C. perfringens* Enterococci	Fecal coliform bacteria *C. perfringens*	Fecal coliform bacteria *E. coli* *C. perfringens*
Shellfish consumption evaluation	Fecal coliform bacteria[b]	-	Fecal coliform bacteria

[a] Fecal coliform bacteria are recommended here because current water quality standards are based on them. If these standards change to include only enterococci, it may still be useful to measure fecal coliform bacteria to maintain continuity with historical databases.

[b] U.S. EPA and National Oceanic and Atmospheric Administration (NOAA) currently are co-sponsoring research into the application of the enterococci and *E. coli* indicators for shellfish harvesting waters.

U.S. EPA has not recommended the use of E. coli for marine and estuarine waters.

Laboratory analyses of fecal coliform bacteria are conducted using one of two most probable number (MPN) techniques (i.e., EC and A-1) or using a membrane filtration (MF) technique. The statistical reliability of the MF technique is greater than the MPN procedures (APHA 1985b). However, factors such as turbidity may reduce counts for the MF technique (Berger and Argaman 1983). Thus, the results generated by the MF and MPN techniques may not be directly comparable, and their inconsistent use by Puget Sound organizations limits comparisons of data gathered by different agencies.

ENTEROCOCCI

Enterococci are streptococcus bacteria indigenous to the intestines of warm-blooded animals. U.S. EPA recommends their use as indicators of fecal pollution in recreational waters because of the previously mentioned limitations of fecal coliform bacteria analysis, and because of the following characteristics:

- Because the concentration of enterococci has a greater correspondence to the incidence of gastroenteritis than do concentrations of E. coli or fecal coliform bacteria, it is a better indicator of public health risk in recreational marine waters (Cabelli et al. 1983).

- Enterococci may die off more slowly in sediments than fecal coliform bacteria (Van Donsel and Geldreich 1971), and therefore be better indicators of sediment contamination.

- Because they are tolerant to high salinity, enterococci are of particular value in analysis of marine waters (Coler and Litsky 1976).

- Taxonomic identification of streptococcus bacteria can be undertaken easily (e.g., API biochemical strips) and, unlike the situation with fecal coliform bacteria, can reveal the kinds of mammalian pollution (e.g., humans, livestock). This advantage arises from the fact that particular kinds of mammals harbor characteristic species of streptococcus bacteria (e.g., S. bovis in cattle).

- Techniques of gene fingerprinting (DNA analyses) have been undertaken using streptococcus bacteria and can more positively link bacteria in the environment to identical bacteria found in source effluents, thereby confirming the source(s) of contamination. Although not suitable for routine monitoring surveys, genetic analysis may be useful in certain research applications.

CLOSTRIDIUM PERFRINGENS

C. perfringens is consistently associated with fecal wastes and provides a usable, state-of-the-art marker for delineating the deposition and/or movement of sewage particulates that is more reliable than the traditional coliform bacteria indicators (Emerson and Cabelli 1982). *C. perfringens* is recommended for water, sediment, and tissue because it is present in wastewater at concentrations of 10^3–10^4 per 100 mL (Fujioka and Shizumura 1985), and because its resistance to chlorination and environmental factors closely resembles that of enteric viruses (Bisson and Cabelli 1980).

LABORATORY PROCEDURES FOR RECOMMENDED BACTERIAL INDICATORS

Recommended laboratory procedures for the bacterial indicators listed in Table 3 are summarized in Table 4.

TABLE 4. RECOMMENDED LABORATORY PROCEDURES FOR BACTERIAL INDICATORS

Test Organisms	Laboratory Procedures		
	Water	Sediment	Tissue
Fecal coliform bacteria	MPN tubes using A-I broth (APHA 1985b) (fecal coliform bacteria/100 mL)	MPN tubes using A-I broth (APHA 1985b) (fecal coliform bacteria/100 mL)	MPN tubes using EC broth (APHA 1985a) (fecal coliform bacteria/100 mL)
Fecal coliform bacteria/*E. coli*	mTEC (DuFour et al. 1981) (*E. coli*/100 mL)	-	-
Enterococci	mE (Levin et al. 1975) (enterococci/100 mL)	-	-
C. perfringens[a]	MPN tubes using iron milk (St. John et al. 1982) (*C. perfringens*/100 mL)	MPN tubes using iron milk (St. John et al. 1982) (*C. perfringens*/100 g)	MPN tubes using iron milk (St. John et al 1982) (*C. perfringens*/100 mL)

[a] Two laboratory techniques are available for *C. perfringens*: mCP by membrane filtration for water (Bisson and Cabelli 1979) and sediment (Emerson and Cabelli 1982), and iron milk tubes using MPN techniques (St. John et al. 1982). The latter method is recommended (pending any comparative data) because the procedure is simpler and less costly.

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

Laboratory and analytical QA/QC procedures are discussed in detail in U.S. EPA (1978) and APHA (1985b). Special problems exist in microbiological analyses because analytical standards, known additions, and reference samples generally are not available. However, a minimum QA/QC program should include:

- Ten percent of the total number of samples analyzed in duplicate
- Ten percent of the total number of samples split and analyzed by two or more laboratories
- Sterile distilled water transported to the field, transferred to a sample bottle, and processed routinely to ensure samples were not contaminated during collection and transport
- Repeated sampling at one site during varying conditions (e.g., tides, weather) to evaluate variability in the field.

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