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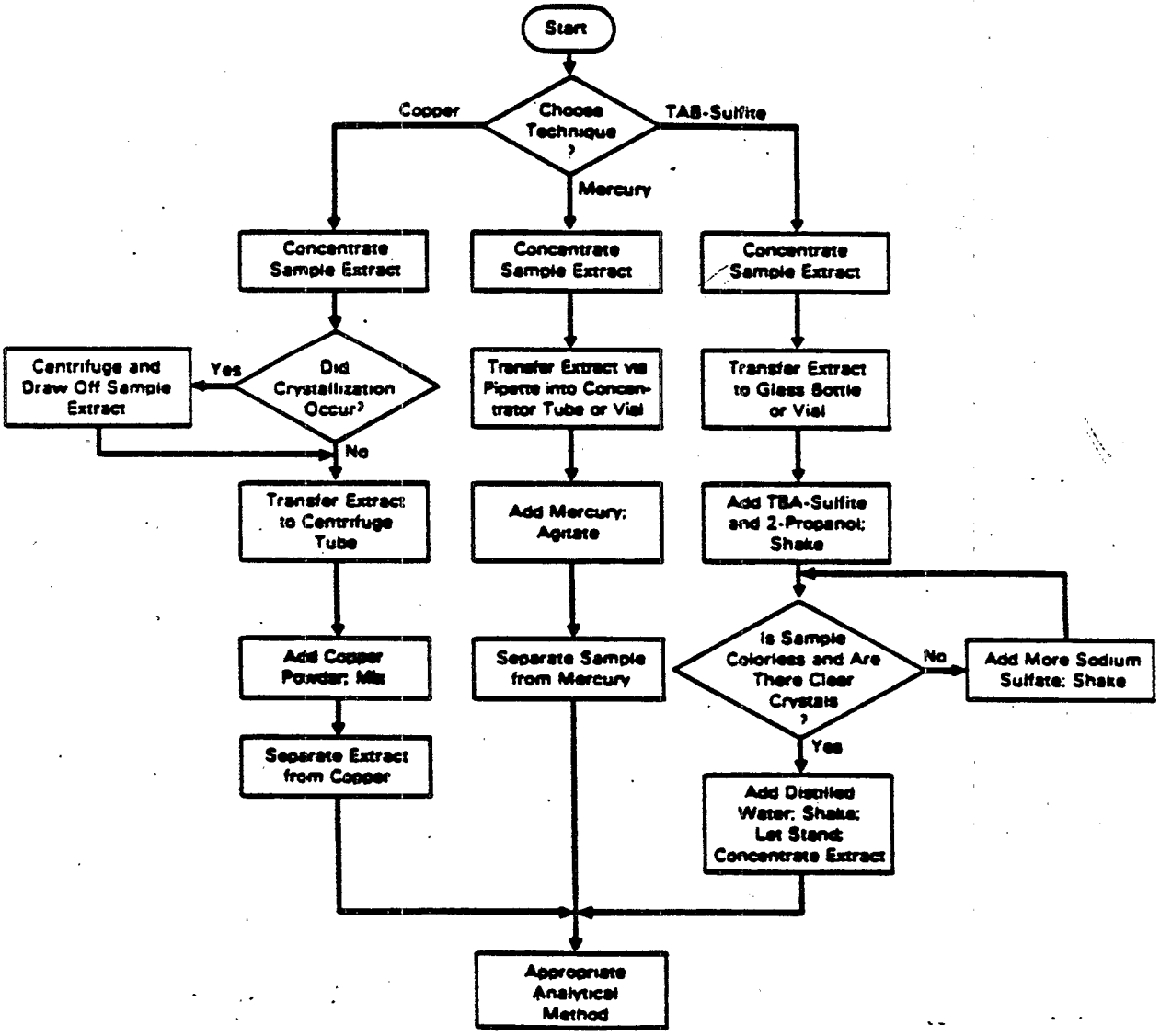
GUIDANCE FOR SAMPLING OF AND ANALYZING FOR ORGANIC CONTAMINANTS IN SEDIMENTS



GUIDANCE FOR SAMPLING OF AND ANALYZING FOR ORGANIC CONTAMINANTS IN SEDIMENTS

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Prepared by:

Christina E. Cowan, Robert G. Riley
Battelle
Pacific Northwest Laboratories
Richland, Washington

For:

U.S. Environmental Protection Agency
Criteria and Standards Division
Washington, D.C.

Submitted by:

BATTELLE
Washington Environmental Program Office
Washington, D.C.

ABSTRACT

Since May 1985, the Criteria and Standards Division of the U.S. Environmental Protection Agency has been pursuing the development of a method for establishing numerical sediment quality criteria. In anticipation of such a method for nonpolar organic contaminants, this report describes recommended procedures for sample collection, preservation, preparation, and analysis to obtain consistent and comparable data for validating and exercising this method.

To ensure that the samples are of high quality, pre-collection planning and preparation are critical. Among the pre-collection activities are preparing the sampling plan, including quality assurance/quality control (QA/QC) plan; choosing the appropriate sampling device; choosing the appropriate method for locating the sampling station; and collecting and preparing the sampling equipment and containers. During collection, accurate and detailed records must be kept of all activities and deviations from those described in the sampling plan. Two types of samples are collected: one for organic carbon and dry weight analysis and the other for contaminant analysis. All samples must be refrigerated or frozen until analyzed.

Analysis of the samples should be conducted only by laboratories that are experienced in applying analytical methods that meet minimum QA/QC requirements. All measurements are to be reported on a dry weight basis by drying the sample for at least 16 hours at 70°C. The method recommended for total organic carbon analysis is dry combustion, using an inductive furnace. Sample preparation and analysis involve Soxhlet extraction of the sediment, cleanup of the extract, and subsequent analysis by gas chromatography/mass spectrometry and/or gas chromatography equipped with electron capture, halogen-specific, or flame ionization detectors. The choice of system depends on instrument availability, individual preference, and class(es) of compounds targeted. Cleanup procedures are described for removing paraffinic, polar, and biogenic materials and sulfur that could interfere with the analysis.

Data derived from implementing the above approaches can then be used to calculate the organic carbon normalized concentration of the contaminant for comparison with the sediment criterion value.

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1.0 INTRODUCTION

Since May 1985, the Criteria and Standards Division of the U.S. Environmental Protection Agency (EPA) has been pursuing the development of a method for establishing numerical sediment quality criteria. Sediment quality criteria are needed because in some freshwater and saltwater sediments around the country, the concentrations of organic and metal contaminants are elevated above background levels (Bolton et al. 1985) and appear to impact the benthic communities associated with those sediments. Furthermore, national water quality criteria alone do not sufficiently ensure that aquatic ecosystems will be protected consistent with the provisions of the Clean Water Acts of 1977 and 1987. Thus, methods are being developed to establish sediment quality criteria for nonpolar organic contaminants [e.g., polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated pesticides] and for metals.

In anticipation of such a method for establishing numerical criteria for nonpolar organic contaminants, recommended procedures are described in this report for collecting sediment samples and for measuring the concentration of nonpolar organic contaminants and associated ancillary parameters in sediments. The first part of this report is intended as a guide to understand and choose the appropriate sample collection and analysis methods. The available methods have been reviewed and those most endorsed by the scientific community for generating quality data on the chemistry of environmental samples are described. The appendixes contain detailed step-by-step descriptions of the methods that enhance or depart from our recommended reference documents. We encourage the adoption of these methods, because they will ensure consistent data necessary for applying sediment quality criteria for nonpolar organic contaminants and for validating these criteria. Standardization of analytical procedures used by the technical community will also permit quantitative comparison of the contaminant concentrations at different sites, and will present a baseline against which modifications to the methods can be compared.

2.0 DESCRIPTION OF APPROACH FOR ESTABLISHING SEDIMENT QUALITY CRITERIA

Before describing the recommended sample collection and analytical methods, we will summarize the approach being pursued by the Criteria and Standards Division of the EPA. This approach is an adoption and implementation of the Equilibrium Partitioning Approach.

The Equilibrium Partitioning Approach is based on two interrelated assumptions. First, the interstitial water concentration of the nonpolar organic contaminants is controlled by partitioning between the sediment and the water. Thus, the interstitial water concentration can be calculated from the quantity of the sorbent(s) on the sediments and the appropriate sorption coefficients. For nonpolar organic contaminants, research has shown that the major sorbent phase on sediments is the particulate organic carbon (Karickhoff 1981, 1984; Karickhoff, Brown, and Scott 1979; Schwarzenbach and Westall 1981; DiToro, Jeris, and Ciarcia 1985). Because of the strong influence of the particulate or total organic carbon on the contaminant partitioning, the partition coefficient is commonly expressed as normalized to the organic carbon content and called the organic carbon partition coefficient, K_{oc}. The partitioning relationship is described by the following equation:

$$C_s/O.C. = K_{oc}(C_{iw})$$

where C_{iw} is the interstitial water concentration, C_s is the sediment concentration, and O.C. is the organic carbon content.

Second, the toxicity and accumulation of the contaminant by benthic organisms are correlated to the interstitial water concentration of the contaminant. The data of Adams, Kimmerle, and Mosher (1986), Swartz[a] and

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(a) From presentation given at Society of Environmental Toxicology and Chemistry, 8th Annual Meeting, November 9-12, 1987, Pensacola, Florida.

Word[a] support the validity of this assumption. This assumption means that chronic water quality criteria or other toxicological data from water column toxicity tests can be used to establish the no-effect or specific-effect concentration in the interstitial water. Based on this assumption, the specified interstitial water concentration can then be used to calculate the concentration of the contaminant on the sediment that results in this interstitial water concentration. To provide for greater transferability between sites, the concentration on the sediment will be reported as normalized to the organic carbon content.

To apply the sediment quality criteria, the concentration of the nonpolar organic contaminants in the sediment under consideration (normalized to organic carbon content) will be compared to the sediment quality criteria value for the sediment (which is also normalized to the organic carbon content). Thus, the major variables that must be measured in each sediment sample are total organic carbon content (%) and the sediment concentration of the contaminants (ng/g), both expressed in terms of the dry weight of the sediment.

(a) Word, J. Q., J. A. Ward, L. M. Franklin, and S. L. Kiesser. 1987. Evaluation of the Equilibrium Partitioning Theory for Estimating the Toxicity of Nonpolar Organic Compound (DDT) on the Sediment Dwelling Amphipod, Rhepoxynius abronius. Prepared for U.S. Environmental Protection Agency, Criteria and Standards Division. Submitted by Battelle, Washington Environmental Program Office, Washington, D.C.

3.0 SAMPLE COLLECTION, PRESERVATION, AND ANALYTICAL METHODS

The major variables that must be determined in the sediment to validate and promulgate the Equilibrium Partitioning Approach are the concentration of the nonpolar organic contaminant and the organic carbon content. Three activities can markedly influence the quality of the measurements of these variables: 1) sample collection and preservation, 2) sample preparation, and 3) sample analysis. The most important consideration in the conduct of these three activities is the experience of the person assigned to perform the work. Many of the sampling and analytical procedures may need to be modified slightly to accommodate variations in sampling conditions and/or differences in sample matrices. Assigning experienced staff will help ensure that proper judgment is exercised if modifications are required and that that sample integrity and data reliability are maintained. Much of the discussion in the sections that follow is focused on methods for collecting and analyzing sediments and solid wastes as described in recent reports (EPA 1984, 1986; Tetra Tech 1986; MacLeod et al. 1985).

3.1 SAMPLE COLLECTION AND PRESERVATION

This section describes the protocols required to collect an acceptable sediment sample for measurement of the physical and chemical parameters. If conducted improperly, sample collection and preservation procedures can adversely affect sample integrity (chemical and physical properties), thereby affecting the quality of the results of chemical analysis performed on that sample. Thus, it is critical that sediment samples be collected and preserved using standard techniques to avoid potential contamination and matrix disruption. For example, the sample type dictates the type of storage container and the necessary storage conditions (e.g., refrigerated, frozen) to ensure sample stability.

3.1.1 Pre-Collection Planning and Preparation

Many of the activities critical to ensuring that the collected samples are of high quality take place in the pre-collection planning and preparation stage. Careful planning and attention to detail at this stage will result in

a more successful field sampling and will ensure collection of the highest quality sample possible. Pre-collection activities include 1) preparing the sampling plan, including a quality assurance/quality control (QA/QC) plan; 2) choosing the appropriate sampling device; 3) choosing the appropriate method for locating the sampling station; and 4) collecting and preparing sampling equipment and containers. At this stage, arrangements must be made to secure a vessel, if necessary, and to choose and notify the analytical laboratory.

3.1.1.1 Sampling Plan

Before the field sampling is conducted, a complete sampling plan should be prepared that includes all QA/QC procedures to be followed during the field activities. A complete plan should address the following items:

- identification of sampling team and responsibilities of each member
- statement of sampling program objectives
- description of area to be sampled and desired sample locations
- variables to be measured and corresponding container and preservation requirements
- identification of sampling methods, including station positioning techniques, sampling devices, replications
- QA/QC procedures, including sample custody and reporting requirements
- cruise schedule
- health and safety plan
- storage and shipping procedures
- all special equipment required
- contingency plans in the event that problems are encountered during sampling, including location and availability of backup equipment.

The plan should be reviewed and understood by all members of the sampling team before going to the field.

The QA/QC procedures are required to ensure that the environmental samples achieve the highest level of quality possible and to document that level of

quality is achieved. Procedures important to collection and preservation of the sediment samples are discussed briefly in this section, while QA/QC procedures important to sample analysis are discussed in Section 3.2.

All procedures involved in locating the sampling station and in collecting and preserving the samples should be described in detail in the sampling plan or provided in standard operating procedures (SOPs) that are referenced in the sampling plan. The sampling team members should be familiar with the appropriate procedures for their part of the field sampling, as well as the record-keeping requirements associated with each procedure.

One of the most important aspects of a successful sampling program is accurate and complete record keeping. A log book, under the responsibility of the field supervisor, should be dedicated to recording all information on field activities and sampling efforts. The level of detail in the log book should be sufficient to permit an uninvolved party to reconstruct the sampling effort. In the planning stages, procedures should be developed for and lists made of all pertinent information to be recorded. Included in the record books should be the date and time of field activities; names of the field team; station locating procedures; information on the sampling site, including information on any photographs taken; appearance of all samples; information on all modifications to procedures conducted during the sampling cruise; and sample numbers with pertinent information on the quantity and type of sample collected and sample handling procedures. Following standard recording procedures for laboratory record books, all entries in the log book should be made in ink and each page signed by the author and reviewed and signed by the field supervisor. All corrections should be lined out, initialed, and dated.

Chain-of-custody procedures and forms should be prepared that allow for the documentation of the samples and their status at every stage in the process from collection through final analysis and entry of data into the data management system. An example of a chain-of-custody and analysis request form is given in Figure 1. Accompanying these chain-of-custody forms should be waterproof sample labels for each of the sample jars. Further details on chain-of-custody forms and sample records are given in Section 3.1.2.7.

Chain-of-Custody and Analyses Request Form

Project Name/Number _____ Field Supervisor _____

Laboratory Book Identification Number _____ Sample Identification Numbers _____ to _____

General Sample Description _____

Sample Number	Sample Type	Analysis	Collection Date	Collection Time	Remarks

Relinquished by (Signature)	Received by (Signature)	Date/Time	Comments
Relinquished by (Signature)	Received by (Signature)	Date/Time	Comments
Relinquished by (Signature)	Received by (Signature)	Date/Time	Comments
Relinquished by (Signature)	Received by (Signature)	Date/Time	Comments
Dispatched by (Signature) / Date/Time	Received for Lab by (Signature)	Date/Time	Comments
Method of Shipment			Discard Date

Distribution: Original - Accompany Shipment
One Copy - Field Supervisor Files

FIGURE 1. Example of Chain-of-Custody and Analysis Request Form

Sample contamination is a significant concern that needs to be addressed prior to and during sample collection. Among the issues that must be addressed and dealt with in the sampling plan are 1) material used in the sampler and cable, 2) material used in and cleanliness of sample containers and collection equipment, 3) presence of potential sources of airborne contamination, and 4) presence of anthropogenic material. The importance of choosing the material for the sampler and cable to avoid sample contamination will be discussed in Section 3.1.1.2. The presence of unwanted anthropogenic material (e.g., bottles, cans, etc.) in the sample will result in rejection of the sample as described in Section 3.1.2.2. Choice of materials for sample containers and collection equipment, and preparation of these containers and equipment to prevent contamination are described in Section 3.1.1.4. To assess the level of contamination, container blanks (i.e., containers that do not contain samples) should be analyzed periodically. In addition, procedural blanks should also be analyzed periodically to ensure that no contamination occurs during field sampling. Before sampling, all potential sources of airborne contamination (e.g., stack gases, cigarette smoke, dust) and other sources of contamination (e.g., grease from ship winches and cables) should be identified and procedures developed to minimize and assess their effect on sample integrity.

3.1.1.2 Sampler Selection

The most common sampling device used for collecting sediments is the modified Van Veen grab sampler, although a variety of other sampling devices (e.g., box corer) are also used and may be appropriate for sampling under various conditions. When sampling at water depths greater than 200 to 300 m, a box corer usually gives better results than the Van Veen grab sampler. A Smith-McIntyre grab sampler may be best for sampling coarser sediments. The primary criterion that should be used to evaluate a sampling device is that it consistently collects undisturbed sediment to the required depth without contaminating the samples. To do so, the sampler must meet the following criteria:

- Create a minimal bow wake when descending to prevent disturbance of the sediment surface.

- Form a leakproof seal after the sediment sample is taken.
- Prevent excessive sample disturbance while ascending.
- Allow for easy access to the sediment sample.
- Be easily and properly handled under the conditions of sample collection.
- Contain weight adjustment.
- Be constructed of material (i.e., stainless steel, Teflon, Kynar) that will not contaminate the sample.

Although most standard sediment samplers seal adequately when they are purchased, the wear and tear of repeated field use may result in sample leakage. Therefore, the integrity of the sampler should be constantly monitored.

The choice of sampling device also depends on the depth of sample required. The penetration/sample depth of the sampler should be several centimeters greater than the depth of the desired sediment sample. For example, a penetration depth of 4 to 5 cm is recommended for collecting a 2-cm surface sample. The penetration depth of a sampler is influenced by its weight and by sediment composition (e.g., penetration is greatest in fine sediments and least in coarse sediments). To ensure adequate penetration, a sampler that has a means of weight adjustment is recommended.

A 2-cm depth is generally recommended when specifically sampling to evaluate surface sediment chemistry. Although that depth is a somewhat arbitrary designation, it assures that the most recently deposited sediments are collected and that adequate volumes of sediment are collected for analysis. Furthermore, this depth holds the majority of benthic organisms and thus represents the bioactive layer. Accurate depth sampling is accomplished by using calibrated scoops.

In addition, special precautions must be taken in the choice of the cable used to deploy the sampler. The cable can potentially be a source of organic and metal contamination. Because the focus of this document is on collecting samples for nonpolar organic contaminants, the cable cannot be greased. However, stainless steel cable may be used.

3.1.1.3 Selection of Station Location Method

Although a variety of navigation and/or position fixing systems are available, factors such as price, availability, and accuracy must be considered in choosing a system for a survey. Criteria to consider in choosing a positioning system include site-specific factors of the sampling program that require certain levels of accuracy or that will limit the feasibility of certain methods. These site-specific criteria will include 1) physical conditions and topography, 2) equipment required, 3) minimum station separation, 4) station reoccupation, and 5) program constraints such as cost, staffing, and operator experience. Other criteria are the ability to meet the study design requirements and provide the desired degree of precision. The most accurate method that is feasible and available is required when evaluating trends and gradients in sediment quality. The accuracy is considered both in terms of the absolute or predicted accuracy (i.e., method's ability to define a position by latitude and longitude) and by repeatable or relative accuracy (i.e., method's ability to return the user to the same position). Each of the available methods has certain absolute and relative accuracy, as well as availability, in certain geographic locations. Thus, no one method can be recommended. However, in general, electronic positioning methods are more accurate than optical methods. Optical methods are only recommended for shore or near-shore sampling (i.e., within 0.5 km) and would be most appropriate for sampling along urban waterfronts. These methods generally tend to be more labor intensive than electronic positioning methods. Table 1, which is reproduced from Tetra Tech (1986), gives the characteristics and advantages/disadvantages of each of the different positioning methods. Calibration of station positions with different methods may be used to assist in the positioning on future sampling trips and to ensure accuracy. All positioning should be used in conjunction with a fathometer to determine the sampling depth and to ensure that the water is of the proper depth and the bottom has the proper profile (i.e., not too much incline) for operation of the sampler.

Once the positioning method has been chosen, the proper setup, calibration, and operational procedures must be followed to achieve maximum method accuracy. Persons (i.e., one primary and one backup) on the cruise who will be responsible

TABLE 1. Summary of Positioning Methods

Category	Accuracy	Range	Advantages	Disadvantages
Theodolite	10 to 30 s ▲1 m and up	<5 km	Traditional method; inexpensive; high accuracy; successfully applied; restricted areas.	Line of site; two manned shore stations; simultaneous measurements; limits on intersection angles; area coverage; station movement.
EDMI	1.5 to 3.0 cm	3 km without multiple prisms	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	5 to 7 cm	<5 km	Single onshore station; other uses; minimum logistics.	Reflector movement and directionality; prism costs; line of sight; optical or infrared range limitations.
Microwave navigation systems	▲1 to 3 m	25 km	No visibility restrictions; multiple users; highly accurate; radio line of sight.	Cost; multiple onshore stations; logistics, security; signal reflective nulls.
Range-azimuth	0.02° and 0.5 m	<5 km (optical) 30 km (elec)	High accuracy; single station; circular coverage.	Single user; cost; line of sight; signal reflective nulls.
Satellite systems	1 to 10 m	none	High accuracy; single minimum logistics; use in restricted/congested areas; future cost; no shore stations.	Current coverage; initial development cost.

TABLE 1. (contd)

Category	Accuracy	Range	Advantages	Disadvantages
Sextant[(a)]	±10 s ±2 m and up	<5 km	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	±15 m and up	none	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	±0.5°	16 to 72 km	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.

for the station positioning should be identified, and their experience and training with the positioning method documented. To achieve an adequate familiarity with the positioning system, appropriate training or securing of qualified and experienced personnel may be required. Backup methods and their operation should be identified in the sampling plan in the event that the primary system fails.

3.1.1.4 Equipment Preparation

To ensure that all required sampling equipment and supplies are on board at the time of field sampling, a check list of the necessary equipment should be prepared. Backup equipment and spare parts should be included on the list. An example of such an equipment check list is given in Table 2.

All sampling equipment (i.e., siphon tubes, scoops, and sample containers) should be made of noncontaminating material such as glass, stainless steel, or polytetrafluoroethylene (PTFE; e.g., Teflon). All equipment including the Teflon lids of the sample containers should be cleaned and dried before use. The recommended equipment cleaning procedure is

1. Wash with detergent.
2. Rinse twice with tap water.
3. Rinse at least twice with distilled water.
4. Rinse with acetone.
5. Rinse with high-purity methylene chloride.
6. Cap with or wrap in fired-aluminum foil.

All sample jars should be capped with and all other equipment wrapped in fired-aluminum foil between cleaning and sample collection to prevent contamination. The final solvent rinse may be substituted by firing aluminum foil-capped glassware at 450°C for 1 h.

3.1.2 Sample Collection Procedures

After all planning has been completed and all necessary equipment located and prepared, the actual sample collection can take place. The sampling procedures will follow those outlined in the sampling plan. Activities included in the sample collection are 1) locating the sample stations, 2) establishing criteria for acceptance of samples, 3) mixing and compositing samples, and

TABLE 2. Sample Equipment Check List

Sediment sampler with spare parts

Station locating equipment

Sample bottles with lids (cleaned)

Mixing bowls and spatula, if necessary (cleaned)

Methylene chloride and foil (for cleaning equipment in field) if necessary

Shipping containers

Dry ice and packing materials

Waterproof labels for sample bottles

Tape for sealing shipping containers

Chain-of-custody and analysis request form

Shipping forms, including "FRAGILE" and "THIS END UP" labels, and custody seals

Siphon tubes

Sampling scoops (cleaned)

Map

Field log book

Indelible ink pens

4) collecting samples. During sample collection and handling, all potential sources of airborne contamination (e.g., stack gases, cigarette smoke, dust) and other potential sources of contamination (e.g., grease from ship winches and cables) should be identified and procedures followed to minimize their effect.

3.1.2.1 Locating Sampling Station

For all samples that are collected, the location of the sampling station must be determined and recorded in the log book. Accurate navigation is essential to ensure that the stations can be plotted and, if additional sampling

is required, reoccupied. Records that should be kept are 1) positioning method and equipment used, 2) names of responsible persons and their duties, 3) location of equipment on board the sampling vessel, 4) modifications in methods or equipment used from those described in the sample plan, and 5) data on the calibration procedures and frequency of calibration. In addition, the occupied stations should be recorded in the log book, and plotted and numbered on the most accurate and up-to-date map of the area. Such maps can be obtained from the U.S. Geological Survey and National Ocean Survey.

3.1.2.2 Operation of Sampler and Criteria for Sample Acceptance

To minimize twisting of the sampler and to ensure proper contact of the sampler with the bottom, the sampling device should be attached to the cable using a ball bearing swivel. The sampler should be lowered through the water column at a controlled rate of approximately 1 ft/s and never allowed to free fall. Free falling could result in premature triggering of the device, excessive bow wake, or improper contact with the sediment surface. Ideally, the sampler should only gently contact the sediment with minimal disturbance and be forced into the sediment only by the weight of the sampler. After the sample is collected, it should be raised slowly from the bottom at a controlled speed of approximately 1 ft/s. To minimize swinging of the sampler when it breaks the surface, the sample vessel should head into the waves. When the sample is brought to the surface, the outside of the sampler should be carefully rinsed with clean water 1) to remove any material on the outside that could contaminate the sample during removal and 2) to permit the sampler to be visually inspected to determine the sample acceptability. The sampler should be secured immediately after it is brought on board to avoid sample tipping, spilling, or disturbance. Excessive swinging of the sampler, striking the vessel, or sampler tipping could result in unacceptable sample disturbance.

If the sample fails any of the following acceptance criteria it should be rejected and another sample taken:

- Sampler is not leaking.
- Desired penetration depth is achieved.

- Sediment surface is flat and does not show signs of disturbance or washout.
- Sample surface is not pressed against the top of the sampler.
- Overlying water is present.
- Overlying water is not turbid.
- Anthropogenic material is not evident (i.e., bottles, cans, etc.).

3.1.2.3 Sample Collection

After the sample is determined to be acceptable, information on the sample should be recorded in the field log book (Section 3.1.2.7).

Before sediment samples are taken for analysis or compositing and mixing, the overlying water must be removed from the sampler by slowly siphoning it off near one side of the sampler. Care should be taken to ensure that the sediments are not disturbed, and that fine-grained surficial sediment and organic matter are not lost while removing the overlying water. Once the overlying water is removed, the sediment can be sampled. To prevent contamination during sample collection, samples should be taken only from the center portion of the sampler to avoid potential contamination from contact with the sampler. In addition, the samples should only come in contact with the cleaned sampling equipment and should not be touched with hands that are not gloved. The samples are placed either in the appropriate sample container or in a stainless steel bowl for compositing and mixing.

3.1.2.4 Mixing of Samples

When removing subsamples for different chemical analyses of the same sediment sample or when combining samples from several sediment grabs to provide sufficient material for analysis, the sample should be thoroughly mixed. The samples can be composited and mixed by transferring them to a dry, solvent-rinsed stainless steel bowl and stirring with a clean stainless steel spoon or spatula until achieving homogeneous color and texture. The bowl and all utensils used for mixing should be changed after each sample or at least solvent rinsed with methylene chloride between uses and covered with foil to prevent airborne or other contamination. The compositing, mixing,

and subsampling should be completed as soon as the samples are collected. However, if a clean room or clean area is not available, then compositing and mixing should not be attempted due to the potential for contamination, but should be completed as soon as possible at the laboratory or other clean facility.

3.1.2.5 Total Organic Carbon and Dry Weight Sample

A minimum of 25 g of sediment sample should be collected in either a glass or plastic container that has been properly cleaned.

3.1.2.6 Contaminant Sample

The sediment sample to be used for analyzing semivolatile compounds, including the nonpolar organic contaminants, should be collected in 240-mL or larger, wide-mouth glass jars with Teflon-lined screw lids. The sample jar must be properly cleaned to prevent contamination of the sample. The sediment sample should be at least 200 g (wet weight). Filling the wide-mouthed jar approximately three-quarters full will ensure obtaining at least this amount of material.

3.1.2.7 Sample Custody Information

After the sample has been collected and stored in the appropriate container, all relevant data pertaining to its collection should be documented in the field log book. Information to be documented should include the following:

- unique sample number
- station location
- date of collection
- depth of water
- gross characteristics, including texture, color, presence of organisms, presence of debris, presence of oily sheen, and odor
- gross characteristics of the vertical profile of the sediment, including changes in characteristics and presence and depth of a redox potential discontinuity layer

- penetration depth of sampler
- depth of sample
- comments on sample quality.

Chain-of-custody procedures and forms should be prepared that allow for the documentation of the samples and their status at every stage in the process from collection through final analysis. An example of a chain-of-custody and analysis request form was given in Figure 1. In addition, waterproof labels containing the sample number, preservation techniques, date and time of collection, location of sample, and signature of the collector should be affixed to each bottle. All writing on these labels should be done with indelible ink.

3.1.3 Sample Preservation and Shipping

Immediately after collection, the samples should be refrigerated (4°C) or placed in shipping containers with dry ice and stored in the dark. Samples should be analyzed within 7 days; however, if the analysis cannot be performed within 7 days, the samples should be frozen to -20°C or to -80°C, if possible. Freezing is required to reduce the potential for microbial activity. Care must be taken with frozen samples to prevent container breakage by leaving headspace for the water to expand. Usually, this is accomplished by freezing the containers at an angle rather than in an upright position. Appropriate handling times have not been established for frozen sediment, although 6 to 12 months are generally considered to be acceptable. Although freezing may alter the sediment matrix, Tetra Tech (1986) presents data that suggest that the effects are minimal.

If possible, all samples should be delivered to the analytical laboratory as soon as sampling is completed to ensure that the samples are analyzed within 7 days. If sample delivery to the laboratory is delayed, then storage procedures described previously must be followed and documented. If the samples contain hazardous materials, guidance for shipping can be found in U.S. Department of Transportation (1984). Procedures that should be followed in preparing the samples for shipping or transportation are as follows:

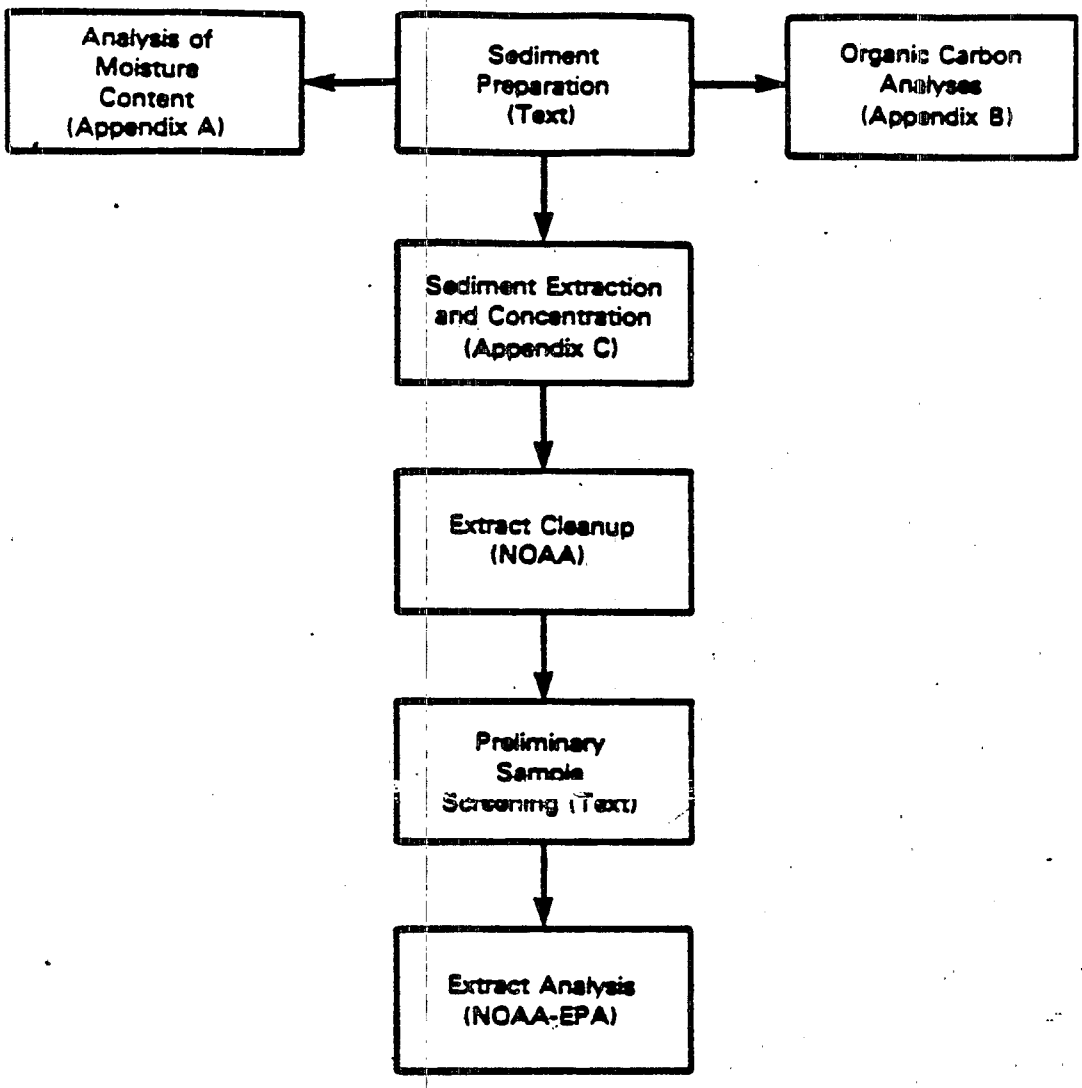
- Containers should be durable and be able to withstand rough treatment during shipping.

- Samples should be tightly packed in shipping containers with dividers and, depending on the shipping time, the space between the bottles filled with packing material or dry ice.
- The original chain-of-custody and analysis request form should be enclosed in protective packaging and placed within the shipping container. Copies of the form should be retained.
- After the samples and forms have been placed in the shipping container, a custody seal, and "FRAGILE" and "THIS END UP" labels should be placed on the outside of the shipping container.
- If the samples must be shipped to a distant city, carriers that provide tracking of shipments and delivery receipts should be used to confirm that the shipment was delivered as required and to serve as additional chain-of-custody information. All shipping charges should be prepaid by the shipper to prevent delay in shipping.
- Notice of receipt of the samples should be received from the analytical laboratory.

3.2 ANALYTICAL PROCEDURES

Before analyzing the sediment sample, the analytical laboratories must be evaluated to determine that they can perform the desired analyses within established guidelines. The review should include an evaluation of the laboratory's instrument capability and the level of staff experience. In addition, the program manager will need to establish minimum QA/QC requirements with the laboratory before the work is done. Evaluation procedures should include analysis of standard reference materials and analysis of replicate samples to establish the quality of a laboratory's analytical capability (e.g., accuracy and precision of their data). Based on the review of data from the available laboratories, the most appropriate analytical laboratory can be selected. The minimum QA/QC requirements should be consistent with those established by the EPA's Contract Laboratory Program.

Figure 2 depicts the general approach that is recommended for the determination of the water and carbon content of the sediments, and for the



EPA = U.S. Environmental Protection Agency
NOAA = National Oceanic and Atmospheric Administration

FIGURE 2. General Approach to Sediment Preparation, Extraction, and Analysis

isolation, characterization, and quantification of nonpolar organic compounds in the sediments. For nonpolar organic compounds, our recommendation is based on a selection process that emphasized the following criteria:

- state-of-art level of the methods and analytical techniques
- ability of the methods to address the analysis of all compounds of interest (Table 3)
- extent of method(s) validation and documentation
- availability of the information on the methods to the user.

Based on a review of the available methods and application of these criteria, we recommend that the standard analytical procedures published by the National Oceanic and Atmospheric Administration's National Analytical facility (MacLeod et al. 1985), referred to as the NOAA method, be used as the main resource for guidance on isolation and analysis of nonpolar organic contaminants from sediments. The NOAA (MacLeod et al. 1985) method is chosen because it satisfactorily meets all of the criteria. The EPA has published several methods for analyzing environmental samples that contain the compounds of interest (see Table 3); however, these methods do not meet one or more of the above criteria. The EPA 600- and 1600-series methods (EPA 1984) are not recommended, because they are specifically directed toward analysis of the compounds in water only, and extraction procedures for sediments are not adequately addressed. The EPA solid waste methods (EPA 1986) are not recommended, because they are not presented in an easily followed, single-analysis scheme from sediment preparation through analysis; they contain too many options to permit the final results to be compared; and they recommend the use of reagents or approaches that are not currently considered appropriate or state of the art. Methods that apply to each of the steps in the general scheme are described in detail in Appendixes A through D and in MacLeod et al. (1985). Recommended departure from the methods in the NOAA report (MacLeod et al. 1985) for sediment dewatering and extraction of large and wet sediment samples are described in Appendix C. Because the NOAA report does not provide a procedure for quantification by

TABLE 3. Nonpolar Organic Priority Pollutants[a]

Low-Molecular Weight PAH

- Naphthalene
- Acenaphthylene
- Acenaphthene
- Fluorene
- Phenanthrene
- Anthracene

High-Molecular Weight PAH

- Fluoranthene
- Pyrene
- Benzo(a)anthracene
- Chrysene
- Benzofluoranthenes
- Benzo(a)pyrene
- Indeno(1,2,3-c,d)pyrene
- Dibenzo(a,h)anthracene
- Benzo(g,h,i)perylene

PCBs

Pesticides

- DDT, DDD, DDE
- Aldrin
- Chlordane (technical mixtures and metabolites)
- Dieldrin
- Heptachlor and metabolites
- Lindane
- Aldrin and metabolites

(a) From Table 1, Section 307, Clean Water Act of 1977

gas chromatography/mass spectrometry (GC/MS), EPA method 8270 (EPA 1986) or EPA Method 1624 (EPA 1984) may be used until a validated approach is developed.

Implementation of the analysis approach (see Figure 2) begins with general sample preparation. During this step, factors that could adversely affect later steps in the analysis scheme are addressed and minimized. As stated previously, all analyses should be performed within 7 days of collection of

the samples. If the analyses cannot be completed within 7 days, then the sample should be frozen (see Section 3.1.3). All sediment preparation procedures and handling should be similar for the samples used for the dry weight, total organic carbon, and contaminant analyses. Following general sample preparation, subsamples of sediment may be analyzed for dry weight (Section 3.2.1 and Appendix A) and total organic carbon content (Section 3.2.2 and Appendix B). Another subsample of the sediment sample is subjected to organic solvent extraction (Section 3.2.3).

Removal of organic constituents from the sediment is achieved by sample dewatering followed by Soxhlet extraction (Appendix C) or by centrifugation and extraction (MacLeod et al. 1985). A key issue that must be addressed during the extraction step is extraction efficiency, which is affected by the ability of the organic solvent to come into intimate contact with sediment particles. Because extraction efficiency is directly related to the presence of water in the sample, aspects of the methods describe approaches for removal (dewatering with methanol) or containment (addition of sodium sulfate) to enhance extraction efficiency. Internal standards are addressed and recommendations are made on compounds to be used for quantification of selected compound classes. Calibration and spiked blanks are also discussed relative to method integrity. Finally, an approach is recommended for concentrating sample extracts that minimizes contaminant losses.

Concentrated sample extracts are then subjected to the very important step of sample cleanup (MacLeod et al. 1985). The cleanup process involves subjecting each extract to a series of treatments that selectively remove materials that would interfere with the analysis of the compounds of interest. Interfering materials that are removed during extract cleanup include elemental sulfur, polar compounds (e.g., acids and bases), paraffinic hydrocarbons, lipids, and other biogenic compounds.

After interfering materials have been removed, it is recommended that samples be subjected to a preliminary screening by gas chromatography to obtain a qualitative measure of sample complexity and the range in contaminant concentration. Information derived from the screening analysis will assist the researcher in determining sample dilution or concentration requirements, in

selecting the appropriate detector, and in determining the extent to which mass spectrometry may be required for chemical characterization and quantification.

3.2.1 Dry Weight Determination

Dry weight of the sediment is determined on an unfrozen sample of the sediment by heating a sample of known weight (5 to 10 g) overnight (or at least 16 hours) at 70°C. The analysis procedure is shown in Figure 3. A drying temperature of 70°C, rather than 105°C, is chosen to provide consistency between the dry weight basis used to report the results of the organic carbon and contaminant analyses, because drying to 105°C could result in loss of some of the semivolatile organic compounds that are targeted for analysis. Before drying, all bulk objects (e.g., sticks, leaves, and rocks) are removed and the sample homogenized. Preparation procedures should be similar to those used in the total organic carbon and contaminant analysis. After the dried sample is allowed to cool in a desiccator, the sample is weighed. A desiccator is used to prevent reincorporation of moisture into the sample during cooling. The dry weight is determined using the following formula:

$$\% \text{ dry weight} = 100 \times \frac{\text{g of dry sample}}{\text{g of sample}}$$

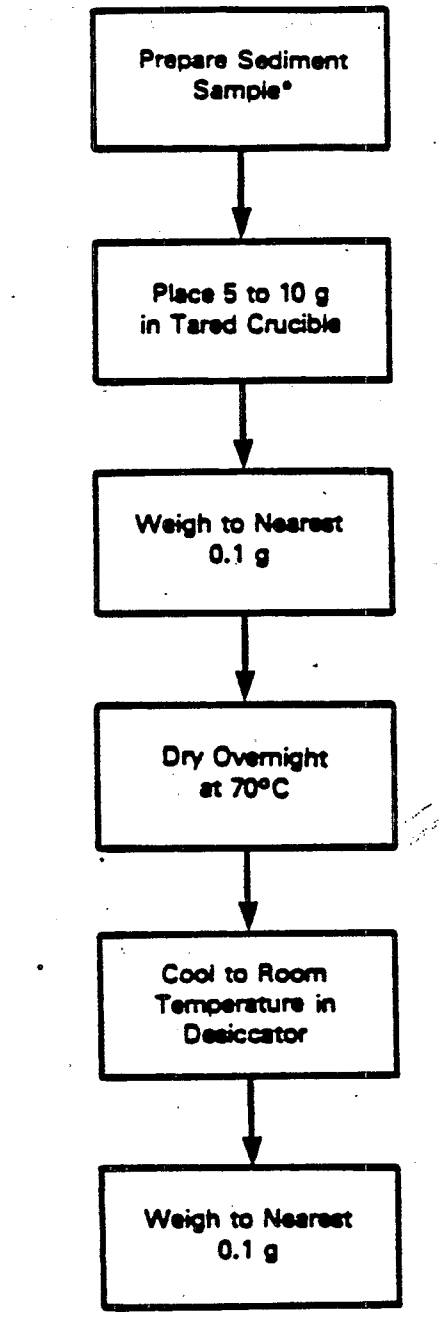
The details of the procedure are described in Appendix A.

The QA/QC procedures and clean laboratory practices should be followed to ensure accuracy of the analyses. The analytical balance should be inspected and calibrated on a preassigned schedule. Desiccators should be checked frequently for proper sealing and for replacement of desiccant to prevent moisture from accumulating during cooling. At least 10% of the samples should be analyzed in duplicate.

The dry weight should be reported as percent to at least 1 decimal place (i.e., 0.1%).

3.2.2 Total Organic Carbon Analysis

Total organic carbon is a measure of the total amount of nonvolatile, partially volatile, volatile, and particulate organic material in a sample.



*Portion of same sample is used for total organic carbon analyses.

FIGURE 3. Flow Chart for Determining the Dry Weight of a Sediment Sample

Many methods for determining the total organic carbon content of soils and sediments have been reported (Black et al. 1965). All the commonly used methods involve either wet or dry combustion of samples and quantitative determination of the organic carbon content of the sediment from the CO_2 that evolves. Quantification methods include volumetric, titrimetric, gravimetric, or conductimetric procedures. With noncalcareous soils, the total carbon evolved is attributed to organic carbon. For calcareous soils, carbonates in the sample that may contribute to the total carbon must be removed before analysis of total organic carbon.

The method recommended for determining the total organic carbon content of the sediment involves dry combustion using an inductive furnace as described in Tetra Tech (1986) and shown in Figure 4. The method is equally applicable to a resistance furnace; however, the inductive furnace is recommended, because preheating is unnecessary and the furnace is activated only after the sample is inserted. Before analysis, the sediment sample is prepared in the same manner as the dry weight and contaminant samples. All bulk objects (e.g., sticks, leaves, and rocks) are removed and the sample is homogenized. Because inorganic carbon (e.g, carbonates, bicarbonates, free CO_2) will interfere with total organic carbon determinations, samples are treated with acid before analysis to remove inorganic carbon. The sample is then burned in a purified stream of O_2 and the CO_2 in the effluent gas stream is determined either gravimetrically, using an ascarite tube to collect the CO_2, or conductimetrically, using a thermal conductivity analyzer. The heat in the inductive furnace is provided by electromagnetic radiation. Soil does not heat well by induction; therefore, metal fines, such as cupric oxide, that can be heated to high temperatures by electromagnetic induction are added to elevate the sample temperature (Black et al. 1965). The detailed description of the method is given in Appendix B. Any other method that is substituted should be compared to this method before acceptance.

As with any of the analytical procedures, QA/QC procedures must be followed to ensure accurate, precise, and reliable measurements. Any laboratory chosen for the analysis must keep records of equipment calibration and follow clean laboratory procedures. The analytical balance should be inspected and

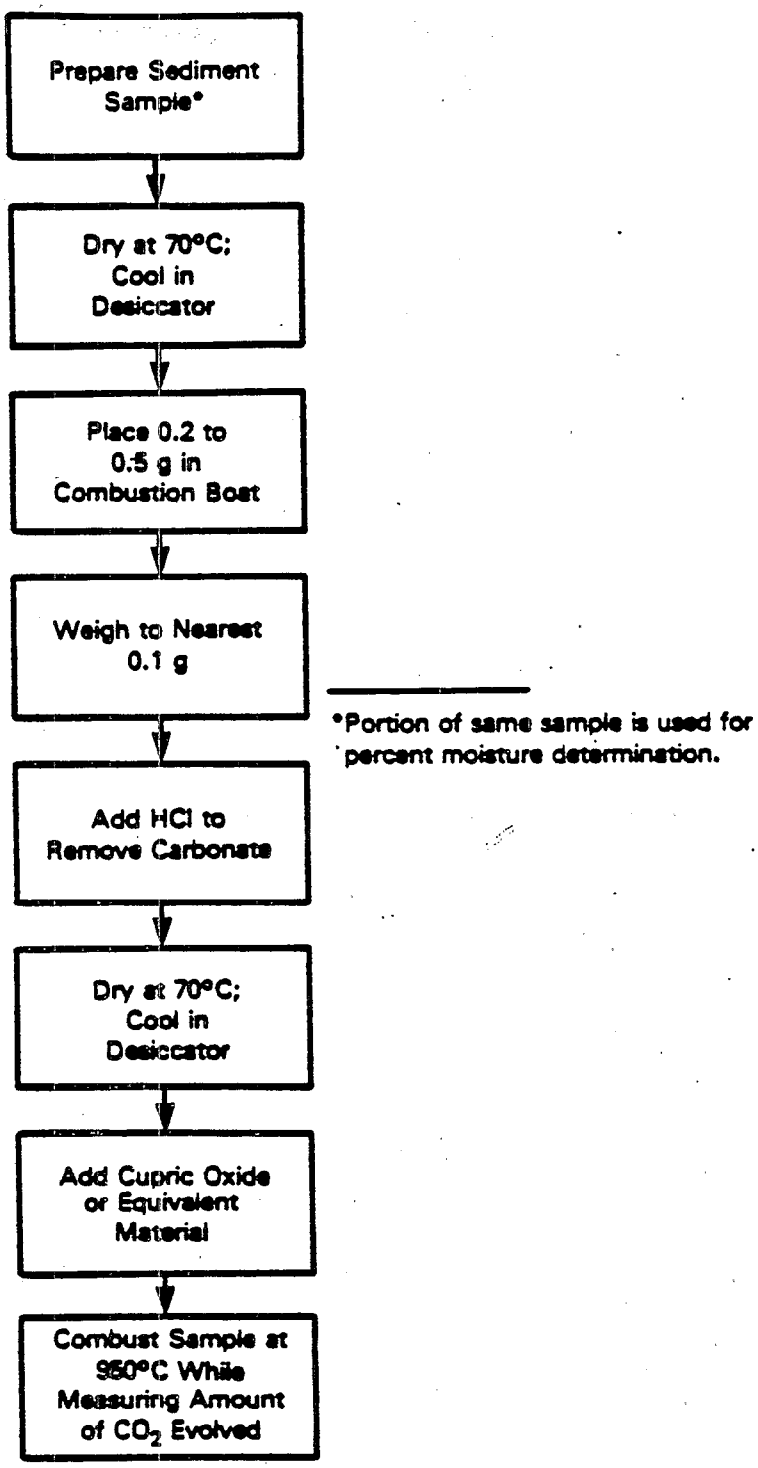


FIGURE 4. Flow Chart for Determining the Total Organic Carbon Content of a Sediment Sample

calibrated on a preassigned schedule. If a carbon analyzer is used, it should be calibrated daily, and a standard reference material should be analyzed at least once with each set of sediment samples. Desiccators should be checked frequently for proper sealing and for replacement of desiccant to prevent moisture from accumulating during drying. In the conduct of sediment total organic carbon analysis, you should 1) use thoroughly homogenized samples, 2) cool all equipment and samples in a desiccator and 3) analyze replicate samples. At least 10% of the samples should be analyzed in duplicate.

The amount of carbon in the sediment sample should be reported as a percent based on the dry weight of the sediment to the nearest 0.1%.

3.2.3 Analysis of Sediments for Semivolatile Priority Pollutants

Procedures for analyzing sediments to determine contaminant concentrations will be limited to those emphasizing semivolatile priority pollutants (see Table 3). These procedures have been developed to detect these pollutants at trace levels in sediments [1 to 50 ng/g dry weight for neutral compounds (e.g., PAHs) and 0.1 to 15 ng/g dry weight of pesticides and PCBs]. As mentioned previously, the recommended procedures are primarily documented in MacLeod et al. (1985). although EPA (1984) is recommended for GC/MS analysis of PAHs.

3.2.3.1 Sample Preparation and Extraction

Figure 5 shows the steps involved in the preparation and extraction of samples. Specific procedures, reagents, materials, and apparatus to perform this step are described in MacLeod et al. (1985) and Appendix C. A sample size of approximately 50 to 100 g (wet weight) of sediment for extraction and a concentrated volume of 0.5 mL are considered adequate to attain the low-level detection limits required for semivolatile organic compounds. However, concentration to as little as 20 µL may be required for characterization of compounds by GC/MS. Smaller sample sizes can adversely affect detection limits, and smaller final volumes can result in excess loss of target compounds because of volatilization. During sample preparation, excess water is decanted from the sample and bulk objects (e.g., sticks, leaves, and rocks) are removed.

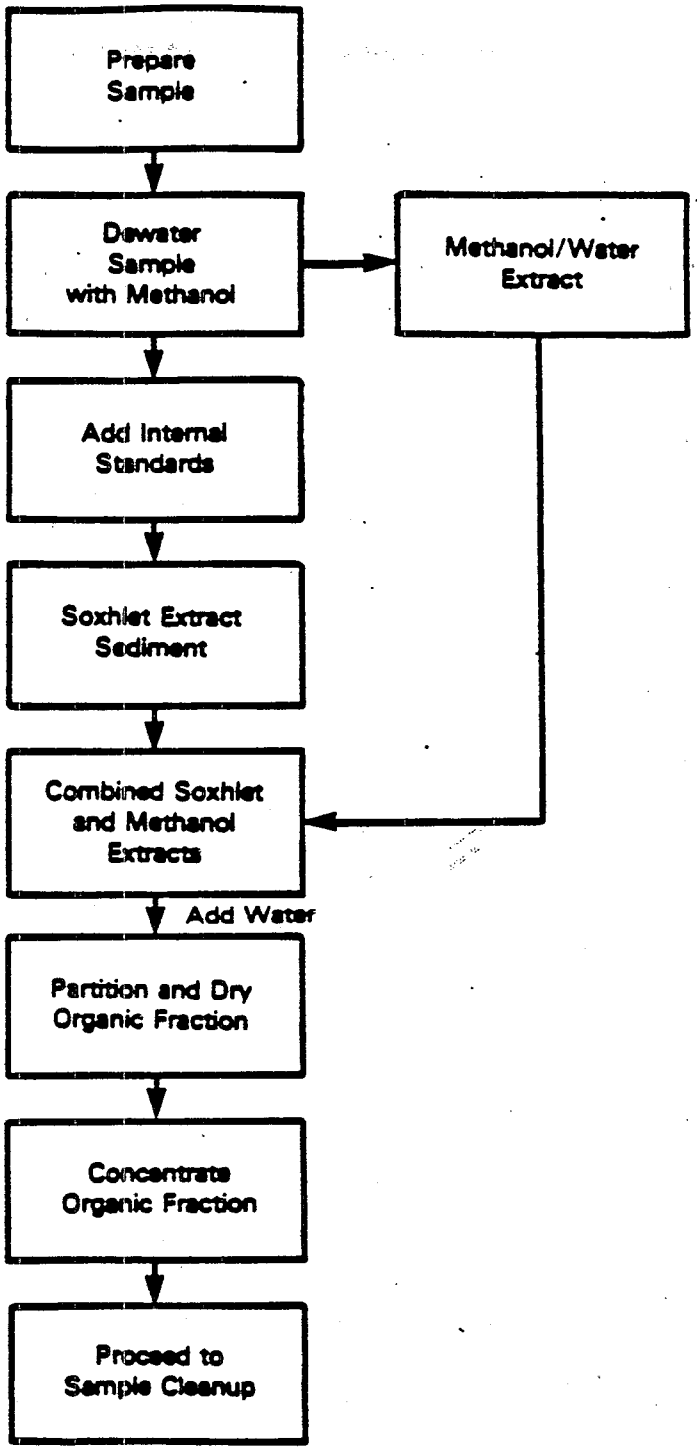


FIGURE 5. Sample Preparation and Extraction

Failure to perform these manipulations could result in poor extraction performance and increase variability in sediment data. It is also important to use a homogenized sediment sample to ensure representativeness of the results.

Prior to Soxhlet extraction, water remaining in the sediment after decanting is removed by contacting the sediment with methanol (Appendix C). In the NOAA method (MacLeod et al. 1985), excessive water is removed by centrifugation, and residual sediment moisture is contained through the addition of anhydrous sodium sulfate to the sediment sample prior to sediment tumbling. The efficacy of the NOAA method with large sediment samples (50 to 100 g) needs to be evaluated.

After sediment dewatering, internal standards are added to the sediments to adjust for analyte losses during sample workup. The standards contain mixtures of deuterated aromatic hydrocarbons or halogenated compounds (Table 4). The internal standard solution for PAHs is prepared in hexane to a concentration of approximately 50 ng/μL for each compound; the internal standard for PCBs and pesticides is prepared in hexane to a concentration of approximately 1 ng/μL. Along with the sediment samples, calibration and spiked blank samples are carried through the extraction procedure to identify and account for potential contamination and losses. Compounds that are recommended for use in the blank samples are given in Tables 5 through 8. The calibration solutions are

TABLE 4. List of Compounds used as Internal Standards[(a)]

Internal Standards for PAHs

Naphthalene-d8
Acenaphthene-d10
Perylene-d12

Internal Standard for PCBs and Pesticides

4.4'-Dibromooctafluorobiphenyl

(a) Adapted from MacLeod et al. (1985)

TABLE 5. List of Compounds used in the PAH Calibration Solution[a]

Hexamethylbenzene
Naphthalene
2-Methylnaphthalene
1-Methylnaphthalene
Biphenyl
2,6-Dimethylnaphthalene
Acenaphthene
Fluorene
Phenanthrene
Pyrene
Benzo(a)anthracene
Chrysene
Benzo(e)pyrene
Benzo(a)pyrene
Perylene
Dibenzo(a,h)anthracene
Naphthalene-d8
Acenaphthene-d10
Perylene-d12

(a) Adapted from MacLeod et al. (1985)

prepared in hexane to a concentration of approximately 5 ng/μL for each compound; the spiked solutions are prepared in hexane to a concentration of approximately 50 ng/μL.

The second step in the analysis is removal of the organic compounds from the sediment samples by Soxhlet extraction. Most Soxhlet extraction procedures reported in the literature use a mixture of solvents that range in polarity. Our recommended Soxhlet extraction solution is benzene/methanol (3:2). However, some laboratories may have safety regulations limiting laboratory worker exposure to benzene, thereby making use of this extraction solution impossible. In this case, we recommend using either methylene chloride/methanol (2:1), methylene chloride/methanol (9:1), or the NOAA method to extraction of the sediment (Figure 6). The EPA method 3540 (EPA 1986) recommends that either toluene/methanol, acetone/hexane, or acetone/methylene chloride mixtures be used. Hunchak and Suffet (1987) showed that acetone and mixtures of acetone

TABLE 6. List of Compounds used in the PCB and Pesticide Calibration Solution[a]

Tetrachloro-m-xylene
Hexachlorobenzene
Lindane (γ-BHC)
Heptachlor
Heptachlor-epoxide
Aldrin
α-Chlordane
Trans-nonachlor
Dieldrin
Mirex
o,p'-DDE
p,p'-DDE
o,p'-DDD
p,p'-DDD
o,p'-DDT
p,p'-DDT
2,4'-Dichlorobiphenyl
2,5,4'-Trichlorobiphenyl
2,4,2',4'-Tetrachlorobiphenyl
2,4,5,2',5'-Pentachlorobiphenyl
2,4,5,2',4',5'-Hexachlorobiphenyl
2,3,4,5,6,2',5'-Heptachlorobiphenyl
2,3,4,5,2',3',4',5'-Octachlorobiphenyl
2,3,4,5,6,2',3',4',5'-Nonachlorobiphenyl
4,4'-Dibromooctafluorobiphenyl

(a) Adapted from MacLeod et al. (1985)

and hexane contain numerous artifacts that can interfere with full scan analysis of environmental samples; therefore, we do not recommend use of any of these extraction solutions.

Following extraction, the methanol/water solution from the sediment dewatering step is combined with the Soxhlet extract and partitioned to obtain the final organic extract. The resulting extract is then dried by elution through a sodium sulfate column and concentrated either by Snyder column or rotary evaporation. We recommend the Snyder column procedure, because it is most effective in removing solvent while minimizing the loss of analyte, although the rotary evaporation method may be faster and less costly. Before

TABLE 7. List of Compounds used in the PAH Spike Solution[a]

Naphthalene
2-Methylnaphthalene
1-Methylnaphthalene
Biphenyl
2,6-Dimethylnaphthalene
Acenaphthene
Fluorene
Phenanthrene
Anthracene
1-Methylphenanthrene
Fluoranthene
Pyrene
Benzo(a)anthracene
Chrysene
Benzo(e)pyrene
Benzo(a)pyrene
Perylene
Dibenzo(a,h)anthracene

(a) Adapted from MacLeod et al. (1985)

the rotary evaporation method is used routinely in a suite of chemical analyses, the recoveries of target compounds must be reported and should be within the acceptable limits as defined by the scientific community for the Snyder column method.

If methylene chloride is used as one of the extraction solvents, then the final step in the extract preparation is solvent exchange with hexane during the final sample concentration step. Removal of methylene chloride is necessary to minimize chromatographic effects during the sample cleanup phase. Final extract volume is targeted at 1 to 2 mL.

3.2.3.2 Sample Cleanup

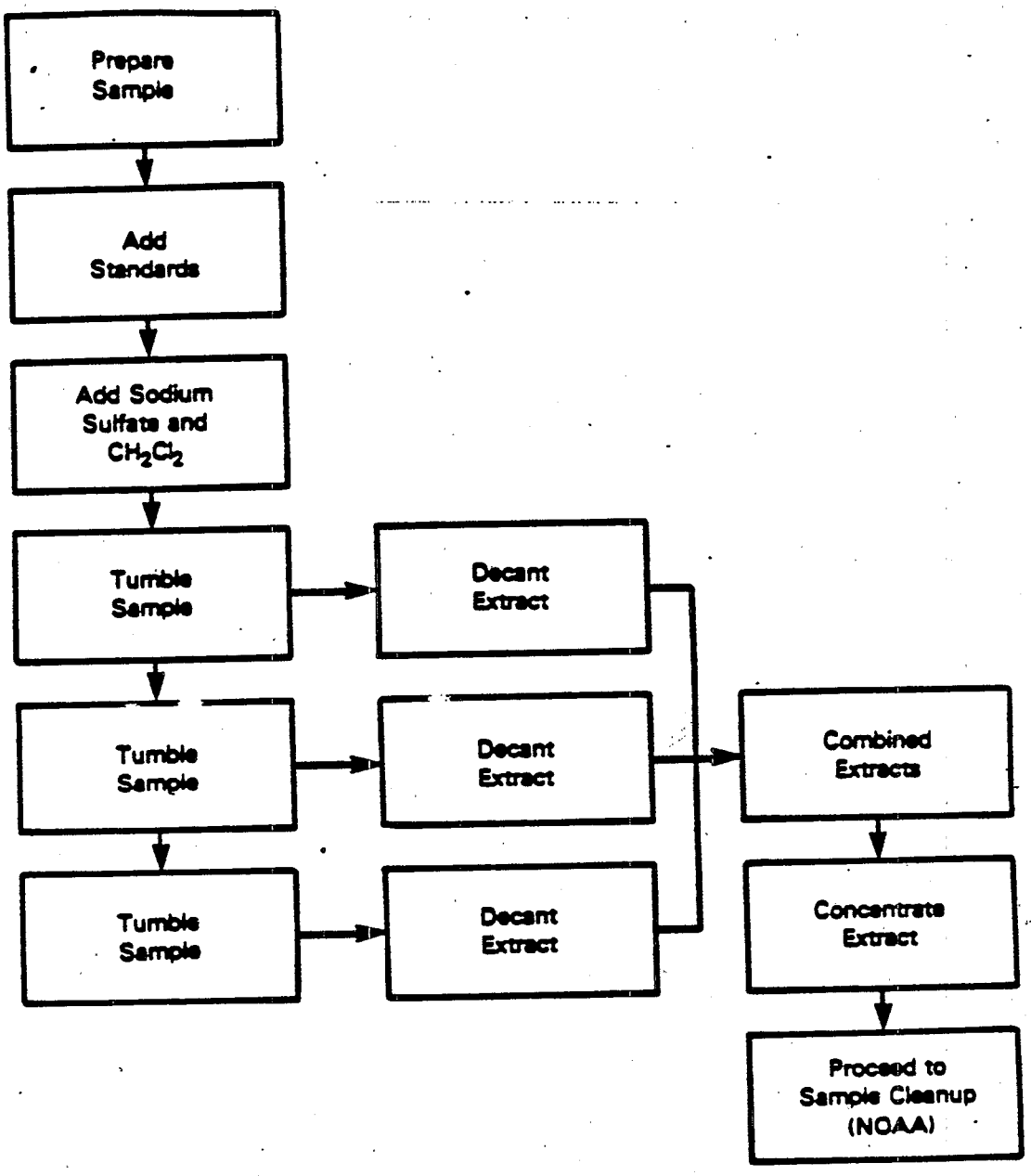
In addition to the nonpolar organic contaminants of interest, the sediment extracts contain a variety of different materials, including polar compounds (e.g., acids and bases), lipids, paraffinic hydrocarbons, inorganic constituents, and other biogenic materials. The objective of the cleanup phase is

TABLE 8. List of Compounds used in the PCB and Pesticide Spike Solution[a]

Hexachlorobenzene
Lindane (γ-BHC)
Heptachlor
Heptachlor-epoxide
Aldrin
α-Chlordane
Trans-nonachlor
Dieldrin
Mirex
o,p'-DDE
p,p'-DDE
o,p'-DDD
p,p'-DDD
o,p'-DDT
p,p'-DDT
2,4'-Dichlorobiphenyl
2,5,4'-Trichlorobiphenyl
2,4,2',4'-Tetrachlorobiphenyl
2,4,5,2',5'-Pentachlorobiphenyl
2,4,5,2',4',5'-Hexachlorobiphenyl
2,3,4,5,6,2',5'-Heptachlorobiphenyl
2,3,4,5,2',3',4',5'-Octachlorobiphenyl
2,3,4,5,6,2',3',4',5'-Nonachlorobiphenyl

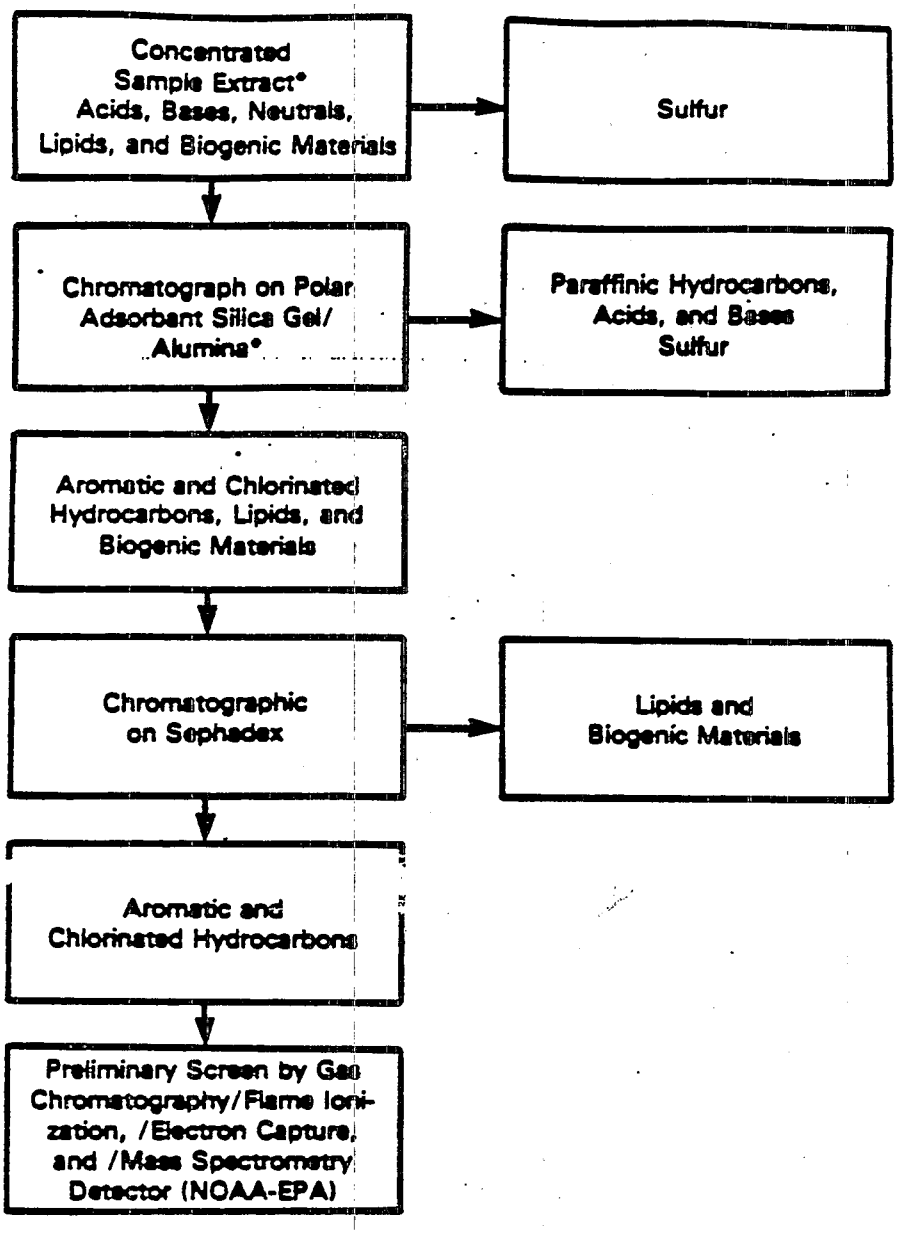
(a) Adapted from MacLeod et al. (1985)

to selectively remove some of these extraneous materials and to reduce/eliminate interferences caused by these materials prior to the chemical analysis phase. Figure 7 depicts, in general terms, the sequence of steps required to clean solvent extracts in preparation for chemical analysis. In the first cleanup step, polar compounds and paraffinic hydrocarbons are removed by subjecting the sample to chromatography on a mixture of silica gel and alumina. The operating mechanism for removal of unwanted compounds in this cleanup step is adsorption. Paraffinic hydrocarbons precede the elution of PAHs, PCBs, and pesticides from the column, and polar compounds are retained by the column. Next, the recovered fraction from the adsorption chromatography step is subjected to chromatography on Sephadex where lipids and other biogenic materials are removed. The primary mechanism for removal of these materials



NOAA = National Oceanic and Atmospheric Administration

FIGURE 6. Sample Preparation and Extraction (NOAA Method)



*Elemental sulfur can be removed from the sample at these stages by several different techniques (Appendix D and MacLeod et al. 1985). If a sample(s) is suspected of having large quantities of sulfur, treatment to remove the sulfur in advance of adsorption chromatography is recommended.

EPA = U.S. Environmental Protection Agency
NOAA = National Oceanic and Atmospheric Administration

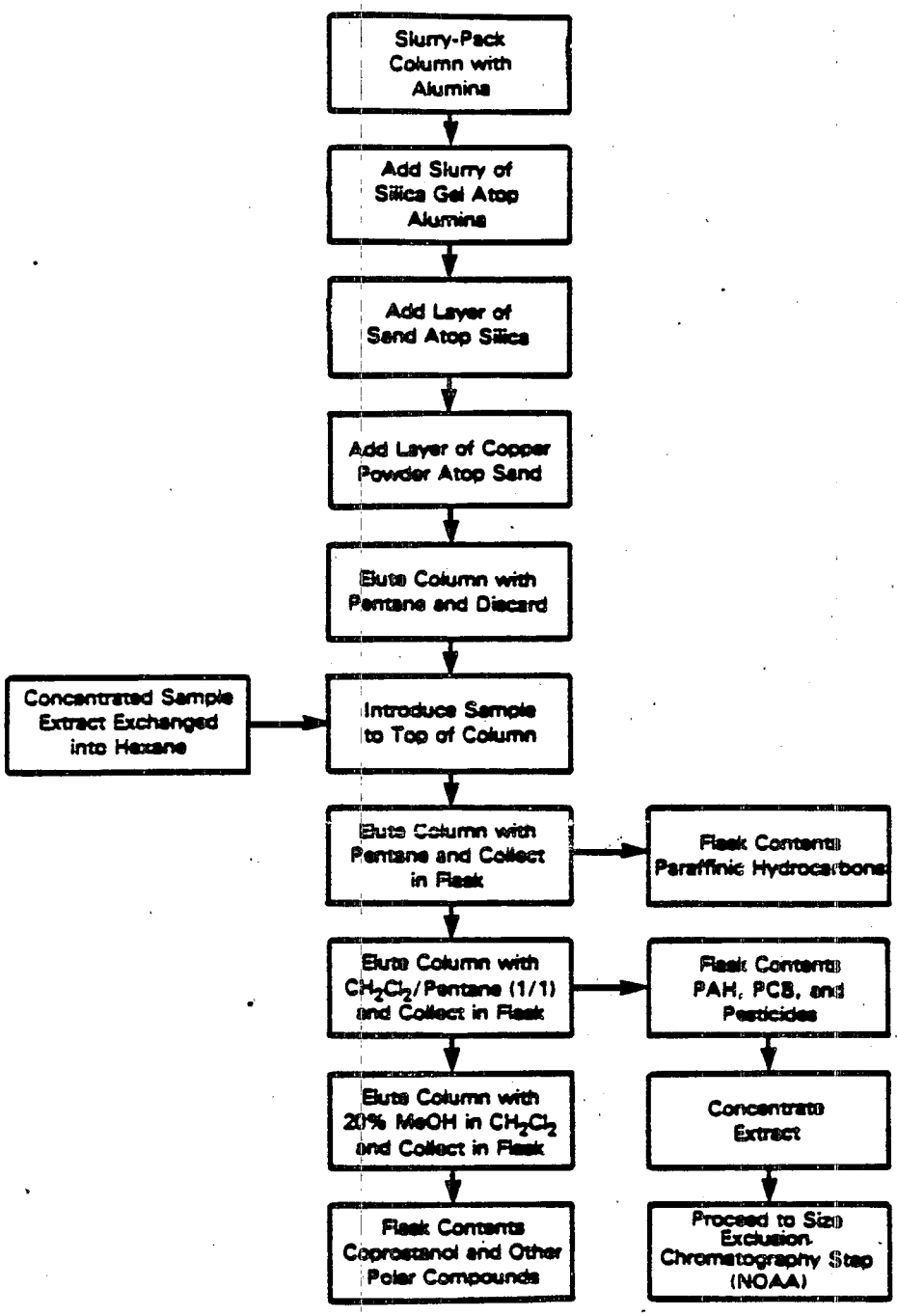
FIGURE 7. General Scheme for Sample Cleanup

in this step in the extract cleanup is size exclusion. High-molecular weight lipid and biogenic materials precede the elution of nonpolar compounds from the column.

Elemental sulfur can also be a major interference in the analysis of nonpolar organic compounds by GC. Specifically, sulfur interferes with the analysis of individual peaks using gas chromatography/electron-capture detection (GC/ECD). Sulfur can be removed from the sample prior to chromatography through reaction with mercury, tetrabutylammonium sulfate, or activated copper. In the case of activated copper, the copper can be added to the top of the adsorption column and sulfur can be removed as part of the sorption chromatography step. For samples that are suspected of containing large quantities of sulfur, it is recommended that the sample(s) be treated prior to adsorption chromatography to remove sulfur using one of the methods given in Appendix D. Although mercury and copper are commonly used to remove sulfur (methods are described in Appendix D), these methods have limitations, including degradation of endrin by mercury and loss of heptachlor in the copper column that preclude their use on certain environmental samples. Following completion of cleanup, the sample is ready for analysis. Each of the cleanup procedures will now be discussed in more detail.

3.2.3.2.1 Cleanup Via Adsorption Chromatography. This section describes in more detail the specific method in which two adsorbents (silica and alumina) are combined to perform the first cleanup step. In this procedure, the recovered extract contains both the aromatic and chlorinated organic compounds of interest. Specific details describing apparatus, reagents, and procedures for this method are described in Section 5 of MacLeod et al. (1985).

The steps depicting the approach are shown in Figure 8. In this approach, slurries of alumina and silica gel are sequentially packed into a glass column. The mixed sorbent is then topped with a layer of acid-washed sand. If sulfur removal is required and sulfur concentrations are suspected to be relatively low (i.e., trace quantities), then a layer of copper powder placed on top of the sand is used to remove sulfur during this chromatographic step. If sulfur concentrations in the extracts are suspected to be relatively high (e.g., sulfur is observed precipitating out of solution), then sulfur cleanup using



NOAA = National Oceanic and Atmospheric Administration
PAH = Polynuclear Aromatic Hydrocarbon
PCB = Polychlorinated Biphenyl

FIGURE 8. Cleanup of PAH, PCB, and Pesticide-Containing Samples via Silica Gel/Alumina Chromatography (Adapted from MacLeod et al. 1985)

one of the methods in Appendix D should be completed before this cleanup step is initiated. The sample concentrate in hexane is introduced to the top of the column and the column sequentially eluted with pentane (fraction contains paraffinic hydrocarbons) followed by a 50/50 mixture of methylene chloride/hexane. The fraction eluting with the latter solvent (designated SA2 in MacLeod et al. 1985) contains the compound classes of interest (i.e., PAHs, PCBs, and pesticides). This fraction is concentrated and then subjected to size exclusion chromatography.

3.2.3.2.2 Sample Cleanup Via Size-Exclusion Chromatography. The second step in the cleanup is size-exclusion chromatography of sediment extracts that have been subjected to adsorption chromatography treatment. The method is designed to remove lipids and other biogenic materials from the sample, further reducing the potential for interference during the screening and chemical analysis steps.

Figure 9 describes the steps that are important to the conduct of the method. Specific details describing apparatus, reagents, and procedures are described in Section 6 of MacLeod et al. (1985). In the first step, the column is packed with a slurry of Sephadex that has been allowed to swell overnight by placing in solvent [cyclohexane:methanol:methylene chloride, 6:4:3 (V:V:V)]. The packed column is then allowed to settle overnight to provide additional time for the solvent to make intimate contact with all surfaces of the porous media and to eliminate air bubbles. If air bubbles persist, pass warm solvent through the column. If this action does not remove the bubbles, then the column will have to be repacked. Prior to column calibration, the height of the Sephadex is adjusted to 26.5 cm. To simulate biogenic/lipid material, a tissue extract that had been subjected to silica gel/alumina chromatography is used to accurately establish its elution profile. The method also makes use of an aromatic hydrocarbon standard (azulene/perylene) in the column's calibration to bracket the elution time of the compounds of interest.

The NOAA report (MacLeod et al. 1985) emphasizes the need to maintain sample solubility. This solubility is achieved by maintaining the sample in a mixed solvent [cyclohexane:methanol:methylene chloride, 6:4:3 (V:V:V)]. Following sample introduction, two lead fractions are eluted from the column

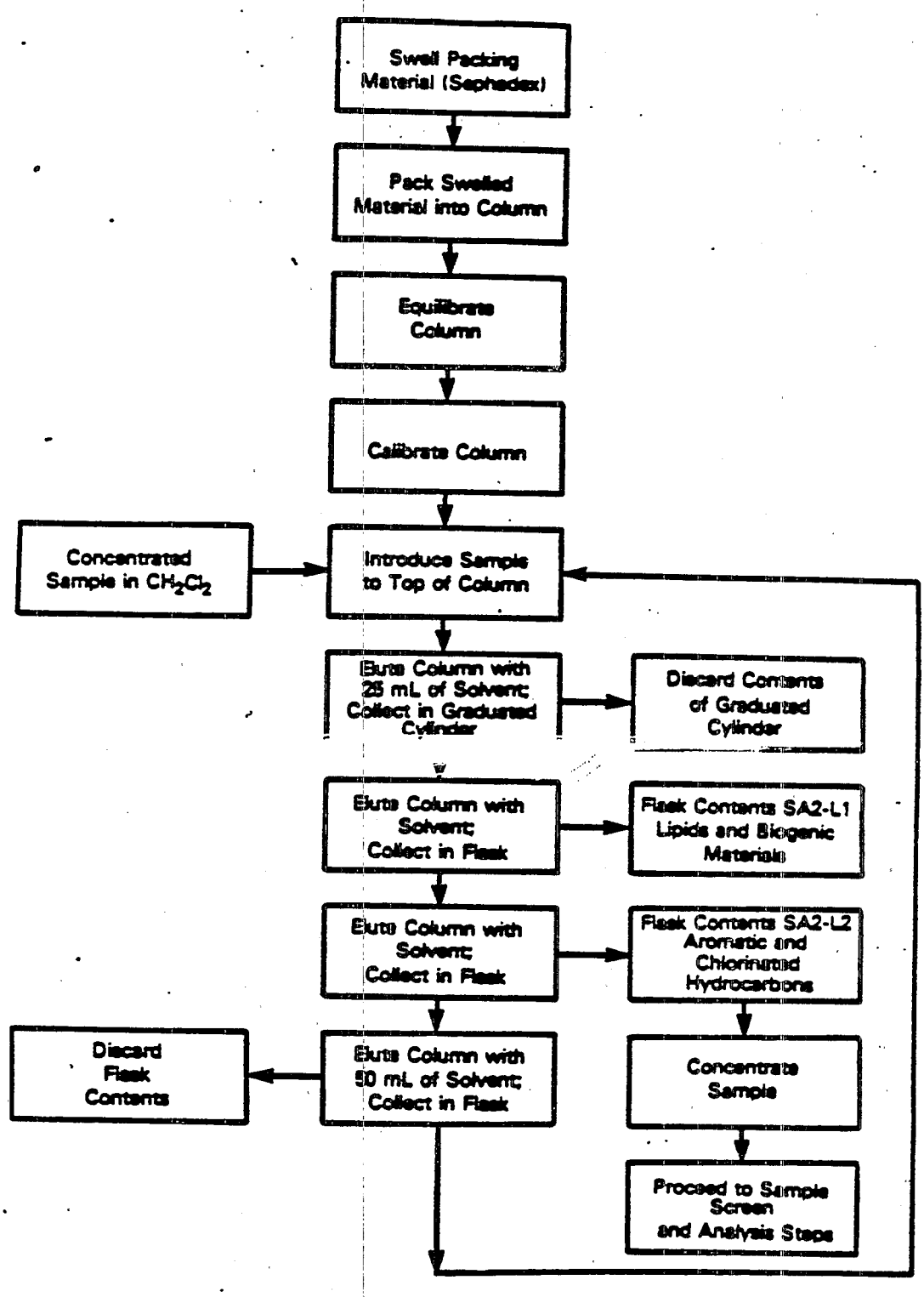
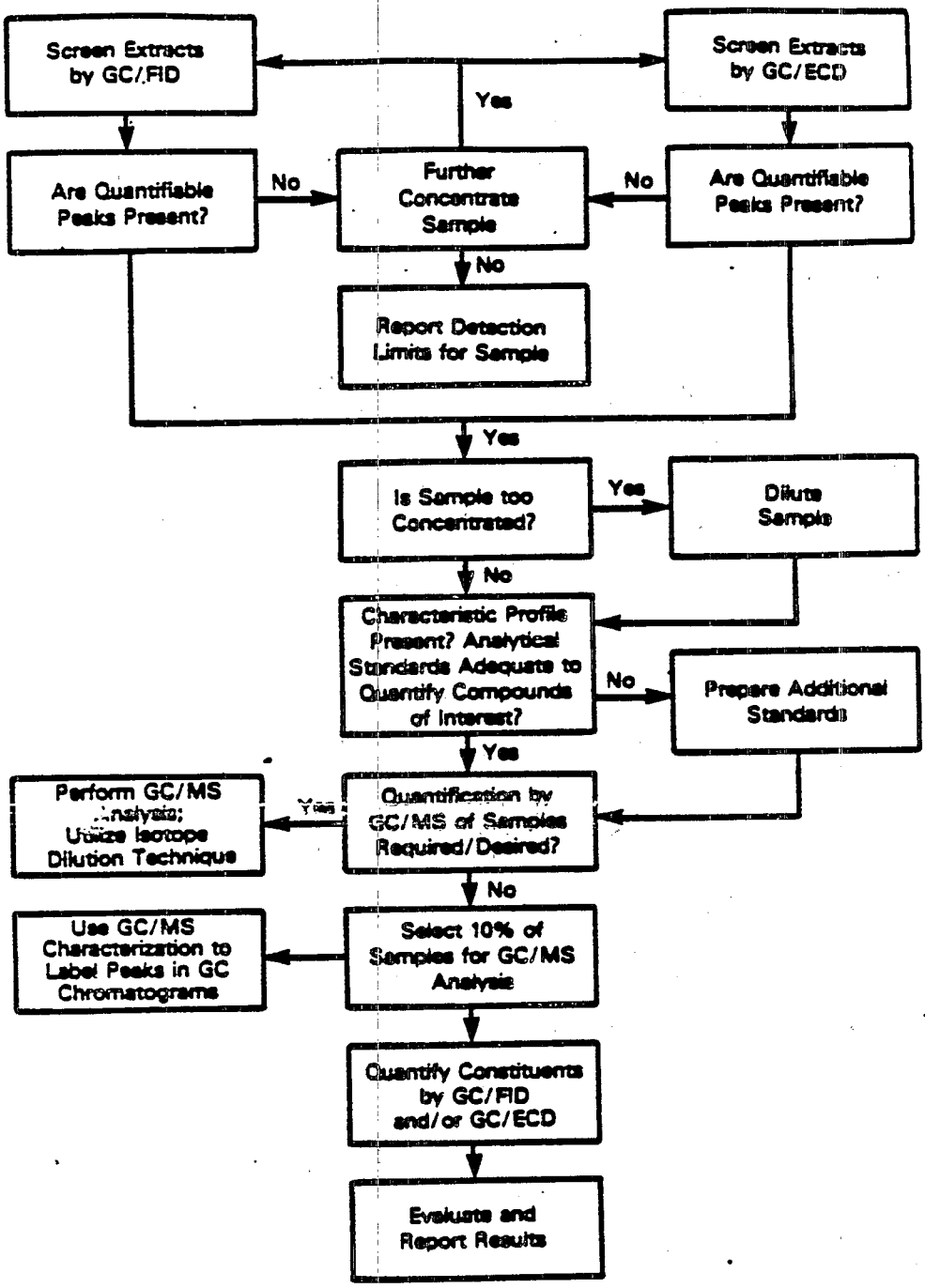


FIGURE 9. Sample Cleanup via Gel Permeation Chromatography (Adapted from MacLeod et al. 1985)

and discarded. These fractions contain lipid and other biogenic materials. A subsequent fraction (Fraction SA2-L2) contains the compounds of interest and is concentrated and subjected to analytical screening and analysis.

3.2.3.3 Analytical Screening and Analysis of Sample Extracts

The decisions facing the analytical chemist once the extracts have been processed through the cleanup steps are governed by experimental needs and by limitations that may be imposed by the make-up of the samples. To address the first point, the researcher will need to have characterization or quantitative data on several classes of compounds in the extract to make decisions on which type of instrument to use in the quantification. Also, concentration of compounds of interest in the extract might be quite low; thereby, taxing to the limit the analyst's skills to obtain the necessary data. Figure 10 depicts the logic for selecting the appropriate analytical tools and for preparing the samples for characterization and quantification of individual constituents by gas chromatography/flame ionization detector (GC/FID), GC/ECD, or GC/MS. In the first step, all samples are screened by GC to determine the relative range in concentration present in all of the samples. Sample volumes at this stage are usually 1 mL. If the researcher is interested in the analysis of PAHs, PCBs, or pesticides, then the screening should include both GC/FID and GC/ECD techniques. Quantitative examination of each chromatogram will allow the analyst to determine whether adjustments in the volume of the sample are required to increase or decrease sensitivity. If increased sensitivity is required, additional sample concentration can be performed. If additional sample concentration (approximately 100 μL) fails to result in detectable peaks (above background), then detection limits will need to be reported for the sample and no additional analysis is required. Alternatively, samples determined to be too concentrated may be diluted by an appropriate amount to ensure accurate quantification. During the qualitative examination of the chromatograms, the researcher may detect familiar patterns characteristic of certain classes of organic compounds. The patterns may include those of PCBs (in the form of specific aroclors) or PAHs (in the form of petroleum). Such information may assist the researcher in analytical standard selection.



GC/ECD = Gas Chromatography/Electron Capture Detector
GC/FID = Gas Chromatography/Flame Ionization Detector
GC/MS = Gas Chromatography/Mass Spectrometry Detector

FIGURE 10. Analytical Screening and Analysis of Samples

Once the researcher has a qualitative picture of the chemistry of each sample, sound decisions on the best quantitative tool can be made. The researcher may have found that the initial screening by GC/FID and/or GC/ECD on some samples provided chromatograms of sufficient quality that the samples will not have to be rerun. If quantification of samples by GC/MS is required or desired, additional adjustments in sample concentration may be required to take into account the lesser sensitivity of the mass selective detector. Although GC/MS has the advantage of more absolute compound identification and increased capability over GC alone to minimize interference/quantification problems, this method is more time consuming and more expensive than GC/ECD or GC/FID methods. Also for most analytes, GC/MS is less sensitive than GC/ECD or gas chromatography/halogen-specific detector (GC/HSD) methods for pesticides and PCBs and GC/FID for PAHs. For example, a sample may have sufficient concentration of PCB to be quantified by GC/ECD, but not by GC/MS. For these reasons, full quantification of all samples by GC/MS may not be required or desired. In this case, the researcher should select 10% of the samples (samples that typify the diversity in complexity of all samples to be analyzed) for GC/MS analysis to confirm the presence of specific compounds, and quantify all samples using either GC/FID or GC/ECD. Samples analyzed by GC/ECD that show the presence of PCBs may also show other peaks from those corresponding to the PCB standards. Representative samples need to be analyzed by GC/MS to identify these peaks and to verify the presence of these other analytes that were indicated by retention time comparisons. In addition, multicomponent peaks are often present (e.g., overlapping PCB and PCB/pesticide combinations) requiring resolution by single ion monitoring. During the quantitative analysis process, the researcher should give serious consideration to using reference standards supplied by the EPA or National Bureau of Standards.

The recommended method for the quantitative analysis of extracts for PAHs, PCBs, and pesticides using GC/FID and GC/ECD is described in detail in Section 12 of MacLeod et al. (1985). Using a capillary column instead of the packed column for gas chromatography is recommended to ensure the resolution of anthracene and phenanthrene, chrysene and benzo(a)anthracene, benzo(b)fluoranthene and benzo(k)fluoranthene, and dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene pairs, and to maximize the resolution of PCB isomers.

Capillary column capability is also required to resolve the deuterated forms of naphthalene, acenaphthene, and perylene that are used as internal standards for PAHs from the nondeuterated compounds in the sediment extracts. To calculate the concentration of chlorinated analytes (i.e., PCBs and pesticides), dibromooctafluorobiphenyl is used as the internal standard for the GC/ECD analysis. The ECD is very sensitive to halogenated compounds; however, the detector requires careful calibration and appropriate use of internal and calibration standards to ensure quality data.

At this time, validated methods for the quantification of nonpolar organic compounds of interest by GC/MS employing isotope dilution techniques are limited to PAH (EPA Method 1624, Rev. B of EPA 1984). If quantification by GC/MS of this class of compounds is required, we recommend EPA Method 1624. For PCBs and pesticides, we recommend the same method with quantification performed employing selective recovery standard(s) (e.g., dibromooctafluorobiphenyl).

3.2.4 Quality Assurance/Quality Control Procedures

To produce analytical results of high quality and reproducibility, QA/QC procedures must be followed to ensure accurate, precise, and reliable measurements. Any laboratory performing these analyses must keep records of equipment calibration, and follow clean laboratory practices. Specific QA/QC procedures include proper preparation of the analytical containers to ensure that no sample contamination will occur from these sources, analysis of all solvents to determine their purity, and calibration and regular maintenance of all equipment. The analytical procedures also include the addition of internal standards to allow for quantification of the analytes of concern and to account for any losses that occur during sample preparation. The compounds used in the internal standards were chosen to ensure representativeness of the compounds of interest and also to ensure that these internal standards will not occur in a GC peak within 0.1 min of the analyte peak. Also included in the analysis are calibration and spiked blank samples to identify and allow the analyst to account for potential contaminant and losses. If the recovery of any internal standard is less than 50%, then the sample must be reanalyzed. A portion of the calibration samples are analyzed in tandem with the extract samples and are used as reference for determining the concentration of the analyte

(Section 3.2.5). Finally, the container and procedural blanks (see Section 3.1.1.1) should be analyzed periodically to assess the level of contamination that occurred during sample collection.

3.2.5 Data Reporting

The final data are reported as the ng of analyte/g of the sediment sample on dry weight basis using the following equation (Section 12 of Macleod et al. 1985):

$$\frac{\text{ng of analyte}}{\text{g of sediment, dry weight}} \times \frac{R_1 \times R_2}{R_3} \times \frac{\text{ng I-Std added}}{\text{sample weight}} \times \frac{100}{\% \text{ dry wt}}$$

where $R_1 = \frac{\text{analyte peak area in extract}}{\text{I-Std peak area in extract}}$

$R_2 = \frac{\text{analyte concentration in reference vial (ng/}\mu\text{L)}}{\text{I-Std concentration in reference vial (ng/}\mu\text{L)}}$

$R_3 = \frac{\text{analyte peak area in reference vial}}{\text{I-Std peak area in reference vial}}$

I-Std = internal standard

%Dry wt = percent dry weight determined using the method in Appendix A.

4.0 DATA CALCULATIONS

After determining the dry weight, organic carbon content, and contaminant concentration, the organic carbon-normalized concentration of the contaminant in the sediment can be calculated and compared with the numerical sediment quality criteria. Because organic carbon is the primary sorbent phase on the sediment and the quantity of the organic carbon affects the toxicity and accumulation of the associated contaminants, a numerical set of criteria that can be used in a cross section of sediment types will be expressed as normalized to sediment organic carbon content. Use the following formula to calculate the organic carbon-normalized concentration of the contaminant:

$$\frac{(\text{contaminant concentration}) \times 100}{\% \text{ organic carbon}}$$

When the contaminant concentration is in units of ng/g and the total organic carbon content is reported as percent, then the units for the organic carbon normalized concentration will be 10^{-7} g of contaminant/g of organic carbon.

5.0 CONCLUSION

Methods have been recommended for collecting sediment samples and for analyzing dry weight, total organic carbon content, and concentration of nonpolar organic contaminants in these samples. The primary concern in applying any of the recommended methods or potential alternatives is that proper QA/QC procedures be followed, and that the performance of the sampling and analysis methods be monitored. If variations in sample matrices require some modifications to the proposed methods, all modifications must be thoroughly documented and their performance must be compared to referenced methods. It is also critical that the procedures be performed only by experienced personnel who follow clean laboratory practices. If these guidelines are followed, the sampling and analysis will meet these criteria and will be useful in applying sediment quality criteria for nonpolar organic contaminants.

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APPENDIX A

METHOD FOR DETERMINING THE DRY WEIGHT OF A SEDIMENT SAMPLE

APPENDIX A

METHOD FOR DETERMINING THE DRY WEIGHT OF A SEDIMENT SAMPLE

A.1 SUMMARY OF METHOD

The sediment sample is homogenized and a portion removed for dry weight determination. The sample is dried overnight at 70°C. After cooling in a desiccator, the weight loss is determined and used to calculate the dry weight of the sample. A flow chart depicting the analytical scheme is shown in Figure A.1.

A.2 EQUIPMENT

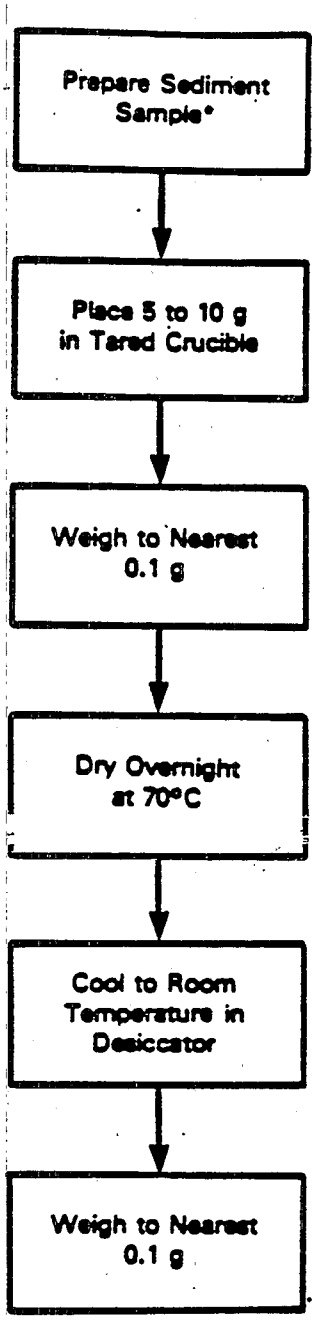
- drying oven capable of sustaining a 70°C temperature
- desiccator
- porcelain crucibles
- apparatus for grinding samples, such as Fisher Mortar Model 155 Grinder, Fisher Scientific Company, Catalogue Number 8-323, or an equivalent brand and model

A.3 EQUIPMENT PREPARATION

- Crucible should be cleaned by heating to 950°C, cooled in a desiccator, and weighed to the nearest 0.1 g.

A.4 SAMPLE PREPARATION

- If possible, the dry weight and other chemical analyses should be performed on fresh sediment samples. However, if the samples must be frozen, then the dry weight should be determined on a frozen sample. Before analysis, the frozen sample should be thawed slowly at room temperature. All bulk materials (e.g., sticks, leaves, and rocks) should be removed from the sample and the sample homogenized to uniform



*Portion of same sample is used for total organic carbon analyses.

FIGURE A.1. Flow Chart for Determining the Dry Weight of a Sediment Sample

texture and color. If fresh samples are used, then overlying water should be discarded before homogenization. If frozen samples are used, water that results from freezing should be incorporated into the sample during homogenization. If the sample will not pass through a 1-mm sieve, it should be processed with the sediment grinder or equivalent equipment to homogeneous texture and color before analysis. The sample preparation procedures should be the same as those used in preparing samples for total organic carbon and contaminant analysis. The sample taken for dry weight determination is one subsample from that collected for total organic carbon analysis.

A.5 ANALYTICAL PROCEDURE

- Place 5 to 10 g of homogenized sample in tared crucible.
- Determine weight to nearest 0.1 g.
- Place crucible with sample in drying oven at 70°C.
- Dry overnight or at least 16 h.
- Cool sample to room temperature in desiccator.
- Determine weight of dried sample to nearest 0.1 g.

A.6 CALCULATION

$$\% \text{ dry weight} = 100 \times \frac{\text{g of dry sample}}{\text{g of sample}}$$

A.7 QUALITY CONTROL

- Crucibles should not be touched with bare hands after cleaning.
- All analytical balances should be inspected and calibrated on a preassigned schedule.
- Desiccators should be checked frequently for proper sealing and for replacement of desiccant.
- 10% of samples should be analyzed in duplicate.

APPENDIX B

METHOD FOR DETERMINING THE TOTAL ORGANIC CARBON CONTENT OF A SEDIMENT SAMPLE

APPENDIX B

METHOD FOR DETERMINING THE TOTAL ORGANIC CARBON CONTENT OF A SEDIMENT SAMPLE

B.1 SUMMARY OF METHOD

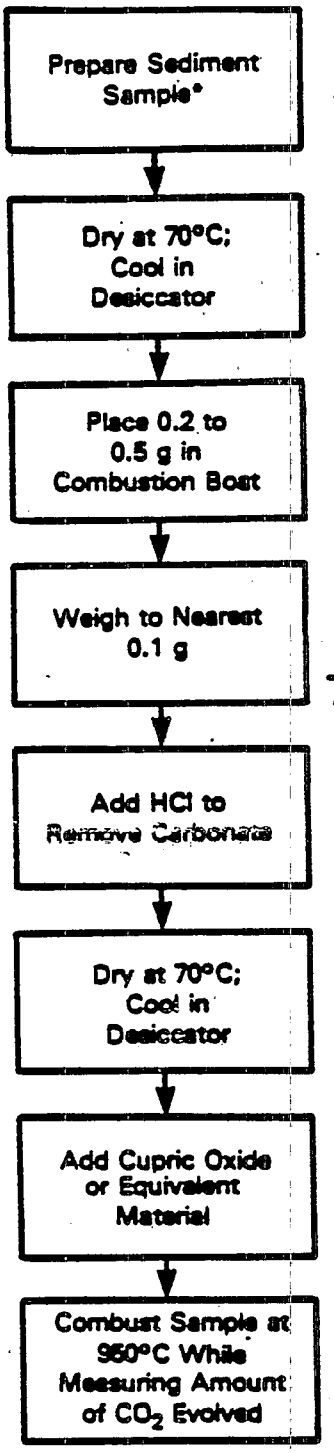
The sediment sample is homogenized and a portion removed for total organic carbon concentration. The sample is first dried at 70°C, then a portion of the sample (0.2 to 0.5 g) is treated with HCl to remove carbonates. The carbonate-free sample is heated to 950°C, and the amount of CO_2 evolved is measured. The analytical scheme is shown in Figure B.1. This method is adapted from Tetra Tech (1986).

B.2 EQUIPMENT

- induction furnace, such as Leco WR-12, Dohrmann DC-50, Coleman CH Analyzer, Perkin Elmer 240 elemental analyzer, Carlo-Erba 1106
- analytical balance with 0.1 mg accuracy
- desiccator
- combustion boats
- 10% hydrochloric acid
- cupric oxide fines or equivalent reference material
- benzoic acid or other carbon source as a standard, if necessary

B.3 EQUIPMENT PREPARATION

- Clean the combustion boats by placing them in the induction furnace at 950°C. After cleaning, the combustion boats should not be touched with bare hands.
- Cool boats to room temperature in a desiccator.



*Portion of same sample is used for percent moisture determination.

FIGURE B.1. Flow Chart for Determining the Total Organic Carbon Content of a Sediment Sample

- Weigh each boat to the nearest 0.1 mg.
- If an ascarite tube is used to capture the CO_2, weigh the tube.
- Set up and calibrate the inductive furnace and associated carbon dioxide analytical equipment according to manufacturer's instructions.

B.4 SAMPLE PREPARATION

A minimum of 25-g samples of the sediment should be collected in a glass or plastic container and stored on ice, if storage times are less than 7 days, or frozen, if storage times are longer. If the samples are frozen, they should be thawed slowly to room temperature before analysis. Homogenize each sample to uniform texture and color.

B.5 ANALYTICAL PROCEDURE

- Transfer 5 to 10 g of sample to a clean container.
- Dry sediment sample to constant weight at 70°C ± 2°C. The drying temperature is kept low to minimize the loss of semivolatile and volatile compounds.
- Cool dried samples to room temperature in a desiccator.
- Grind sample using a mortar and pestle to break up aggregates.
- Transfer 0.2 to 0.5 g of the sample to a cleaned, preweighed combustion boat.
- Determine the sample weight to the nearest 0.1 g.
- Add several drops of 10% HCl to the dried sample to remove the carbonates. Wait until effervescing is completed before adding more acid. Continue adding acid until effervescing no longer occurs when acid is added. The acid should be added slowly and in small quantities to prevent loss of sample due to effervescing.
- Dry the HCl-treated sample to constant weight at 70°C ± 2°C.
- Cool the sample to room temperature in a desiccator.

- Add previously ashed cupric oxide or equivalent reference material to the combustion boat.
- Combust the sample in the inductive furnace at a minimum temperature of 50°C ± 10°C, collecting or measuring the CO_2 evolved.

B.6 CALCULATIONS

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

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

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

A silica trap should be placed in the line before the ascarite tube to prevent moisture from entering the tube. An additional silica trap may also be placed at the exit end of the ascarite tube to trap any water formed by reaction of the CO_2 with the NaOH in the tube.

- If an elemental analyzer is used, the amount of CO_2 will be measured directly. To ensure accuracy, the instrument should be calibrated daily, using the empty boat as a blank for the zero value and at least two standards covering the expected range of the samples. The carbon content is calculated as follows:

$$\text{percent carbon} = \frac{(C)(100)}{B}$$

where C = amount of carbon that is evolved in grams as determined by the carbon analyzer

B = dry weight in grams of the unacidified sample in the combustion boat.

B.7 QUALITY CONTROL

- Crucibles should not be touched with bare hands after cleaning.
- All analytical balances should be inspected and calibrated on a preassigned schedule.
- Desiccators should be checked frequently for proper sealing and for replacement of desiccant.
- If carbon analyzer is used, it should be calibrated daily with standard reference material.
- 10% of samples should be analyzed in duplicate.

B.8 REFERENCE

Tetra Tech. 1986. Recommended Protocols for Measuring Organic Compounds in Puget Sound Sediment and Tissue Samples. Prepared for Office of Puget Sound, Region 10, U.S. Environmental Protection Agency, Seattle, Washington.

APPENDIX C

SEDIMENT DEWATERING AND EXTRACTION

APPENDIX C

SEDIMENT DEWATERING AND EXTRACTION

C.1 SUMMARY OF METHOD

The method combines attributes of procedures described in Tetra Tech (1986a, 1986b). The sediment sample is dried with methanol, placed in an extraction thimble or between two plugs of glass wool, and extracted using an appropriate solvent in a Soxhlet extractor. The combined extract (methanol and Soxhlet) is subjected to liquid-liquid partitioning. The organic phase is then dried, concentrated, and, as necessary, exchanged into a solvent compatible with the cleanup procedures. The analytical scheme is shown in Figure C.1.

C.2 APPARATUS AND MATERIALS

- Soxhlet extractor - 40-mm I.D., with 500 mL round-bottom flask.
- drying column - 20-mm I.D. Pyrex chromatographic column with Pyrex glass wool at bottom and a Teflon stopcock.

  NOTE: Fritted glass disks are difficult to decontaminate after highly contaminated extracts have been passed through. Columns without frits may be purchased. Use a small pad of Pyrex glass wool to retain the adsorbent. Prewash the glass wool pad with 50 mL of acetone followed by 50 mL of elution solvent prior to packing the column with adsorbent.

- Kuderna-Danish (K-D) apparatus
  - Concentrator tube - 10 mL, graduated (Kontes K-570050-1025 or equivalent); ground-glass stopper is used to prevent evaporation of extracts
  - Evaporation flask - 500 mL (Kontes K-570001-500 or equivalent); attach to concentrator tube with springs

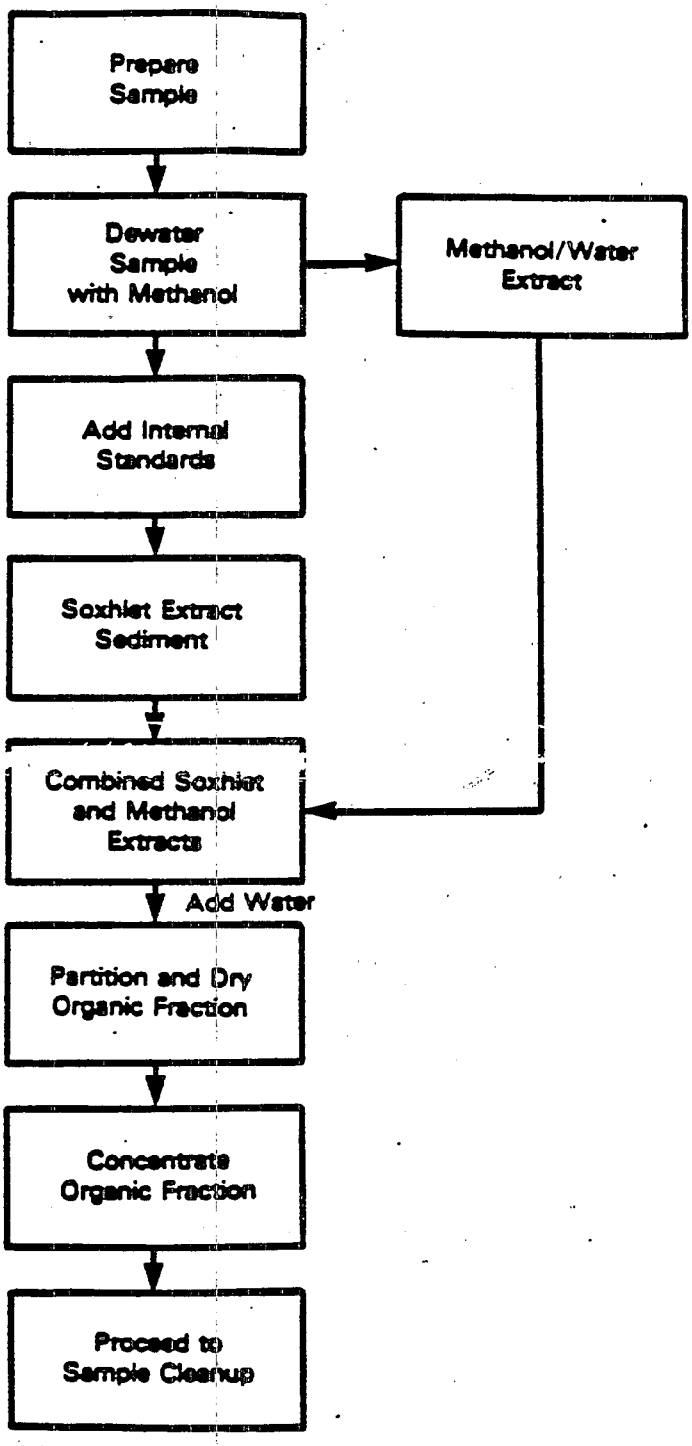


FIGURE C.1. Sample Preparation and Extraction

- snyder column - three-ball macro (Kontes K-503000-0121 or equivalent)
- snyder column - two-ball micro (Kontes K-569001-0219 or equivalent)

- boiling chips - solvent extracted, approximately 10/40 mesh (silicon carbide or equivalent)
- water bath - heated, with concentric ring cover, capable of temperature control (±5°C); the bath should be used in a hood
- vials - glass, 2-mL capacity, with Teflon-lined screw cap
- glass or paper thimble or glass wool - contaminant free
- heating mantle - rheostat controlled
- syringe - 5 mL
- apparatus for grinding - If the sample will not pass through a 1-mm standard sieve or cannot be extruded through a 1-mm opening, it should be processed into a homogeneous sample that meets these requirements. Fisher Mortar Model 155 Grinder, Fisher Scientific Co., Catalogue Number 8-323, or an equivalent brand and model, is recommended for sample processing. This grinder should handle most solid samples, except gummy, fibrous, or oily materials.
- analytical balance

C.3 REAGENTS

- reagent water - reagent water is defined as water in which an contamination is not observed at the method detection limit of the compounds of interest.
- methanol - pesticide quality or equivalent
- extraction solvents - sediment samples shall be extracted using either of the following solvent systems:
  - methylene chloride/methanol 2:1 (V:V), pesticide quality or equivalent

- methylene chloride/methanol 9:1 (V:V), pesticide quality or equivalent
- Benzene/methanol 3:2 (V:V), pesticide quality or equivalent.

- exchange solvent - hexane that is pesticide quality or equivalent.

C.4 SAMPLE PREPARATION

If a fresh sediment sample is used, decant and discard any water layer on a sediment sample. If a frozen sample is used, thaw slowly at room temperature and incorporate overlying water. Mix sample thoroughly, especially composited samples. Discard any foreign objects (e.g., sticks, leaves, and rocks). Homogenize to uniform texture and color.

C.5 ANALYTICAL METHODS

- Sediment Dewatering. Weigh 50- to 100-g sample of sediment to nearest 0.1 g. Mix sample of wet sediment with 50 mL of methanol in a glass centrifuge bottle. The mixture is centrifuged at 163 X gravity (1000 rpm) for 10 min. The supernatant is removed and saved. The methanol wash is repeated and the two supernatants combined. The supernatant solution is filtered through glass wool and the glass wool is rinsed with an additional 10 mL of methanol. The filtered solution is saved. Alternatively the sediment and methanol solution may be placed in an extraction thimble and the methanol removed by draining. A glass wool plug above and below the sample in the Soxhlet extractor is an acceptable alternative for the thimble. The glass wool plug will filter the methanol solution. Save the filtered methanol sample for combining with the Soxhlet extract.
- Addition of Internal Standard Spike. Place dewatered sediment sample in Soxhlet thimble. Add 1.0 mL of the internal standard spiking solution(s) into the sample. Cover the sample with a thin layer of solvent-cleaned glass wool. An internal standard (i.e., a chemically inert compound not expected to occur in an environmental sample) should be added to each sample and the blank sample just prior to

extraction or processing. The recovery of the internal standard is used to monitor for unusual matrix effects, gross sample processing errors, etc. Standard recovery is evaluated for acceptance by determining whether the measured concentration falls within the acceptance limits. Recommended standards for different analyte groups follow; however, these compounds or others that better correspond to the analyte group may be used if past experience warrants.

- Polynuclear aromatic hydrocarbons (PAH) internal spiking solutions: The recommended internal standards are the deuterated compounds of naphthalene-d8, acenaphthalene-d10, and perylene-d12. Prepare the internal standard spiking solution in hexane that contains the compounds at a concentration of approximately 50 ng/μL. The final concentration that is used will depend on the nature of the sample.

- Organochlorine pesticide and polychlorinated biphenyl (PCB) internal spiking solution: The recommended internal standard for organochlorine pesticides and PCBs is p,p' dibromooctafluorobiphenyl. Prepare the internal standard spiking solution at a concentration of 1 ng/μL in hexane. The final concentration that is used will depend on the nature of the sample.

- Extraction. Place 200 mL of the extraction solvent and one or two clean boiling chips into the Soxhlet thimble. Attach the flask to the extractor and extract the sample for 16 to 24 h. Stir the sample in the thimble at least twice (after the second cycle and after approximately 12 h) to prevent solvent channeling. (The glass wool should be removed during stirring and then replaced.) The Soxhlet apparatus should be wrapped up to the condenser with aluminum foil to ensure even heating during cycling. Allow the extract to cool after extraction is completed.

- Liquid-Liquid Extraction. Transfer the cooled extract to a 500-mL separatory funnel. Rinse the Soxhlet flask twice with clean extraction solvent and add this rinse to the extract in the separatory funnel. Add the filtered methanol solution from the dewatering step. Wash

the solvent extract with approximately 100 mL of 50% Na_2SO_4 saturated organic-free water. Collect and store the organic layer. Re-extract the aqueous phase twice with 60 mL of clean hexane and add both extracts to the initial organic fraction. If benzene/methanol was used in the extraction process, the organic layer will be the top phase in the funnel. If one of the methylene chloride/methanol mixtures was used, the organic layer will be the bottom phase.

- Formation of emulsions or precipitates during liquid-liquid extraction should be noted and considered when reviewing the results. The addition of Na_2SO_4 may reduce emulsions; however, if the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimal mechanical technique depends on the sample and may include stirring, filtration of the emulsion through precleaned glass wool, or centrifugation.

- Dry and Concentrate the Extract. Assemble a K-D concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporation flask.

  - Dry the organic layer by pouring it through an anhydrous Na_2SO_4 drying column (approximately 30 cm by 2 cm). Use approximately 30 mL of hexane to rinse the drying column and combine this solution with the dried extract. Collect the dried extract in a K-D concentrator.

  - Add one or two clean boiling chips to the flask and attach a three-ball macro Snyder column. Prewet the Snyder column by adding approximately 1 mL of methylene chloride to the top of the column. Place the K-D apparatus in a hot water bath (15°C to 20°C above the boiling point of the solvent), so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration in 10 to 20 min. At the proper rate of distillation, the balls

of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 min.

- Solvent Exchange. The solvent exchange step is required before the cleanup of the samples if methylene chloride was used in the extraction solution. To perform the solvent exchange, remove the Snyder column, add 50 mL of hexane, and reattach the Snyder column. Concentrate the extract as described in the previous paragraph.

- Calibration and Spiked Standard Solutions. The calibration solutions for PAHs, PCBs, and pesticides are prepared in hexane to a concentration of approximately 50 ng/μL for each of the compounds given in Tables C.1 and C.2. The spiked standard solutions for PAHs, PCBs, and pesticides are also prepared in hexane to a concentration of approximately 50 ng/μL for each of the compounds given in Tables C.3 and C.4. These solutions are subjected to the same analytical procedures as the actual samples starting at the Soxhlet extraction step.

  - The extracts obtained may now be cleaned up to remove interferences and analyzed using the methods in MacLeod et al. (1985; starting at Section 5) or if sulfur cleanup is required by using one of the methods in Appendix D. If cleanup and analysis of the extract will not be performed immediately, stopper the concentrator tube and refrigerate. If the extract will be stored longer than 2 days, it should be transferred to a Teflon-sealed screw-cap vial and labeled appropriately.

C.6 QUALITY CONTROL

- All calibration and spiked standard solutions should be subjected to exactly the same analytical procedures as those used on actual samples.

- Solvents, reagent, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these

TABLE C.1. List of Compounds used in the PAH Calibration Solution[a]

Hexamethylbenzene
Naphthalene
2-Methylnaphthalene
1-Methylnaphthalene
Biphenyl
2,6-Dimethylnaphthalene
Acenaphthene
Fluorene
Phenanthrene
Pyrene
Benzo(a)anthracene
Chrysene
Benzo(e)pyrene
Benzo(a)pyrene
Perylene
Dibenzo(a,h)anthracene
Naphthalene-d8
Acenaphthene-d10
Perylene-d12

(a) Adapted from MacLeod et al. (1985)

materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

- Phthalate esters contaminate many types of products commonly found in the laboratory. Plastics, in particular, must be avoided. Phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Serious phthalate contamination may result at any time if consistent quality control is not practiced.

- Soap residue on glassware may cause degradation of certain analytes. Specifically, aldrin, heptachlor, and most organophosphorous pesticides will degrade in this situation. This problem is especially pronounced with glassware that may be difficult to rinse (e.g., 500-mL K-D flask). These items should be hand-rinsed very carefully to avoid this problem.

TABLE C.2. List of Compounds used in the PCB and Pesticide Calibration Solution(a)

Tetrachloro-m-xylene
Hexachlorobenzene
Lindane (γ-BHC)
Heptachlor
Heptachlor-epoxide
Aldrin
α-Chlordane
Trans-nonachlor
Dieldrin
Mirex
o,p'-DDE
p,p'-DDE
o,p'-DDD
p,p'-DDD
o,p'-DDT
p,p'-DDT
2,4'-Dichlorobiphenyl
2,5,4'-Trichlorobiphenyl
2,4,2',4'-Tetrachlorobiphenyl
2,4,5,2',5'-Pentachlorobiphenyl
2,4,5,2',4',5'-Hexachlorobiphenyl
2,3,4,5,6,2',5'-Heptachlorobiphenyl
2,3,4,5,2',3',4',5'-Octachlorobiphenyl
2,3,4,5,6,2',3',4',5'-Nonachlorobiphenyl
4,4'-Dibromooctafluorobiphenyl

(a) Adapted from MacLeod et al. (1985)

TABLE C.3. List of Compounds used in the PAH Spike Solution[a]

Naphthalene
2-Methylnaphthalene
1-Methylnaphthalene
Biphenyl
2,6-Dimethylnaphthalene
Acenaphthene
Fluorene
Phenanthrene
Anthracene
1-Methylphenanthrene
Fluoranthene
Pyrene
Benzo(a)anthracene
Chrysene
Benzo(e)pyrene
Benzo(a)pyrene
Perylene
Dibenzo(a,h)anthracene

(a) Adapted from MacLeod et al. (1985)

TABLE C.4. List of Compounds used in the PCB and Pesticide Spike Solution[a]

Hexachlorobenzene
Lindane (γ-BHC)
Heptachlor
Heptachlor-epoxide
Aldrin
α-Chlordane
Trans-nonachlor
Dieldrin
Mirex
o,p'-DDE
p,p'-DDE
o,p'-DDD
p,p'-DDD
o,p'-DDT
p,p'-DDT
2,4'-Dichlorobiphenyl
2,5,4'-Trichlorobiphenyl
2,4,2',4'-Tetrachlorobiphenyl
2,4,5,2',5'-Pentachlorobiphenyl
2,4,5,2',4',5'-Hexachlorobiphenyl
2,3,4,5,6,2',5'-Heptachlorobiphenyl
2,3,4,5,2',3',4',5'-Octachlorobiphenyl
2,3,4,5,6,2',3',4',5'-Nonachlorobiphenyl

(a) Adapted from MacLeod et al. (1985)

C.7 REFERENCES

MacLeod, W. D., D. W. Brown, A. J. Friedman, D. G. Burrows, O. Maynes, R. W. Pearce, C. A. Wigren and R. G. Bogar. 1985. Extractable Toxic Organic Compounds, Second Edition. Standard Analytical Procedures of the NOAA National Analytical Facility 1985-1986. NOAA Technical Memorandum NMFS F/NWC-92, Seattle, Washington.

Tetra Tech. 1986a. Analytical Methods for U.S. EPA Priority Pollutants and 301(h) Pesticides in Estuarine and Marine Sediments. Prepared for Marine Operations Division, Office of Marine and Estuarine Protection, U.S. Environmental Protection Agency, Washington, D.C.

Tetra Tech. 1986b. Recommended Protocols for Measuring Organic Compounds in Puget Sound Sediment and Tissue Samples. Prepared for Office of Puget Sound, Region 10, U.S. Environmental Protection Agency, Seattle, Washington.

APPENDIX D

METHODS FOR SULFUR CLEANUP OF EXTRACTS

APPENDIX D

METHODS FOR SULFUR CLEANUP OF EXTRACTS

D.1 SUMMARY OF METHOD

Three techniques for the elimination of sulfur are described: 1) the use of copper powder, 2) the use of mercury, and 3) the use of tetrabutylammonium (TBA)-sulfite. The TBA-sulfite causes the least amount of degradation of a broad range of pesticides and organic compounds, while copper and mercury may degrade organophosphorous and some organochlorine pesticides (Table D.1).

The sample to undergo cleanup is mixed with either copper, mercury, or TBA-sulfite. The mixture is shaken and the extract is removed from the sulfur cleanup reagent. The analytical scheme is shown in Figure D.1.

D.2 APPARATUS AND MATERIALS

- mechanical shaker or mixer, such as the Vortex Genie
- pipettes, disposable, Pasteur-type
- centrifuge tubes, calibrated, 12 mL
- glass bottles or vials - 10 and 50 mL, with Teflon-lined screwcaps.

D.3 REAGENTS

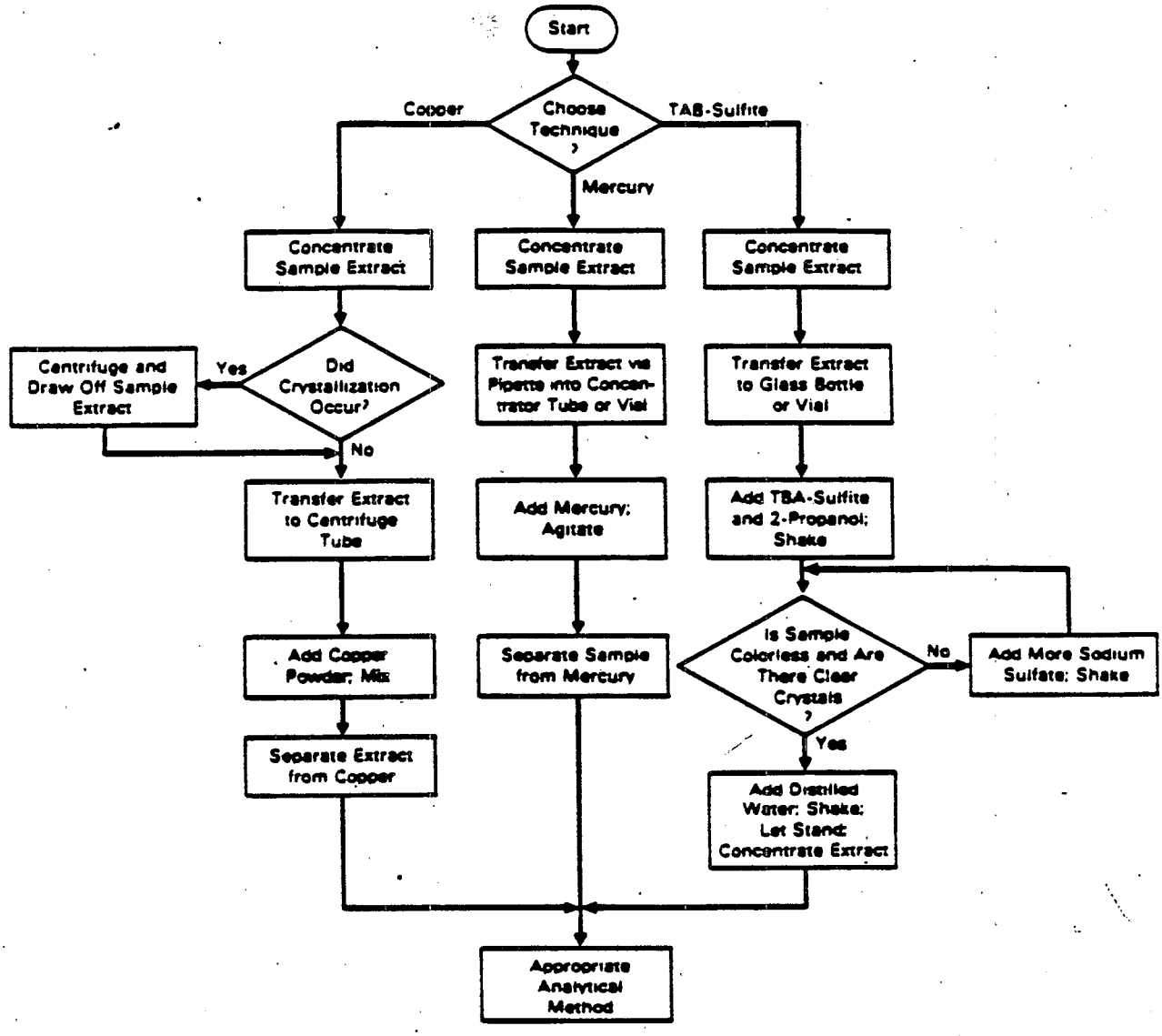
- reagent water - Reagent water is defined as water in which a contamination is not observed at the method detection limit of the compounds of interest.
- nitric acid - dilute.
- acetone, hexane, 2-propanol - pesticide quality or equivalent.
- copper powder - Remove oxides by treating with dilute nitric acid, rinse with distilled water to remove all traces of acid, rinse with acetone, and dry under a stream of nitrogen (copper, fine-grained Mallinckrodt 4649, or equivalent).

TABLE D.1. Effect of Mercury and Copper on Recovery of Pesticides

Pesticide	Percent Recovery[(a)] Using: Mercury	Copper
Aroclor 1254	97.10	104.26
Lindane	75.73	94.83
Heptachlor	39.84	5.39
Aldrin	95.52	93.29
Heptachlor epoxide	69.13	96.55
DDE	92.07	102.91
DDT	78.78	85.10
BHC	81.22	98.08
Dieldrin	79.11	94.90
Endrin	70.83	89.26
Chlorobenzilate	7.14	0.00
Malathion	0.00	0.00
Diazinon	0.00	0.00
Parathion	0.00	0.00
Ethion	0.00	0.00
Trithion	0.00	0.00

(a) Percent recoveries cited are averages based on duplicate analyses for all compounds other than for aldrin and BHC. For aldrin, four and three determinations were averaged to obtain the result for mercury and copper, respectively. Recovery of BHC using copper is based on one analysis.

- mercury - triple distilled.
- TBA-sulfite reagent - Dissolve 3.39 g TBA hydrogen sulfate in 100 mL of reagent water. To remove impurities, extract this solution three times with 20-mL portions of hexane. Discard the hexane extracts, and add 25 g sodium sulfite to the water solution. Store the resulting solution, which is saturated with sodium sulfite, in an amber bottle with a Teflon-lined screw-cap. This solution can be stored at room temperature for at least 1 month.



TAB = Tetrabutylammonium

FIGURE D.1. Analytical Scheme for Removal of Sulfur from Extracts

D.4 SAMPLE PREPARATION

The sample used is the final product from the extraction obtained from the procedures in Appendix C or from Section 7 of MacLeod et al. (1985).

D.5 ANALYTICAL METHODS

- Removal of sulfur using copper
  - Concentrate the sample to exactly 1.0 mL in the Kuderna-Danish (K-D) tube.
  - If the sulfur concentration is such that crystallization occurs, centrifuge to settle the crystals, and carefully draw off the sample extract with a disposable pipette, leaving the excess sulfur in the K-D tube. Transfer the extract to a calibrated centrifuge tube.
  - Add approximately 2 g (to the 0.5 mL mark) of cleaned copper powder to the centrifuge tube. Mix for at least 1 min on the mechanical shaker.
  - Separate the extract from the copper by drawing off the extract with a disposable pipette and transfer to a clean vial. The volume remaining still represents 1.0 mL of extract.

    NOTE: This separation is necessary to prevent further degradation of the pesticides.

- Removal of sulfur using mercury

    NOTE: Mercury is a highly toxic metal and, therefore, must be used with great care. Prior to using mercury, it is recommended that the analyst become acquainted with proper handling and cleanup techniques associated with this metal.

  - Concentrate the sample extract to exactly 1.0 mL.
  - Transfer 1.0 mL of the extract into a clean concentrator tube or Teflon-sealed vial using a disposable pipette.

- Add one to three drops of mercury to the vial and seal. Agitate the contents of the vial for 15 to 30 sec. Prolonged shaking (2 h) may be required; if so, use a mechanical shaker.
- Separate the sample from the mercury by drawing off the extract with a disposable pipette and transfer to a clean vial.

- Removal of sulfur using TBA-sulfite
  - Concentrate the sample extract to exactly 1.0 mL.
  - Transfer the 1.0 mL to a 50-mL clear glass bottle or vial with a Teflon-lined screw-cap. Rinse the concentrator tube with 1 mL of hexane, adding the rinsings to the 50-mL bottle.
  - Add 1.0 mL TBA-sulfite reagent and 2 mL 2-propanol, cap the bottle, and shake for at least 1 min. If the sample is colorless or if the initial color is unchanged, and if clear crystals (precipitated sodium sulfite) are observed, sufficient sodium sulfite is present. If the precipitated sodium sulfite disappears, add more crystalline sodium sulfite in approximately 100-mg portions until a solid residue remains after repeated shaking.
  - Add 5 mL distilled water and shake for at least 1 min. Allow the sample to stand for 5 to 10 min. Transfer the hexane layer (top) to a concentrator tube and use the K-D technique to concentrate the extract to 1.0 mL.
- Complete the cleanup of the extracts by using the method in Section 5 of MacLeod et al. (1985).

D.6 QUALITY CONTROL

- All reagents should be checked prior to use to verify that interferences do not exist.
- The copper must be very reactive, therefore, all oxides of copper must be removed so that the copper has a shiny, bright appearance.

- The sample extract must be vigorously agitated with the reactive copper for at least 1 minute.

D.7 REFERENCES

U.S. Environmental Protection Agency (EPA). 1986. Test Methods for Evaluating Solid Waste. SW-846. Office of Solid Waste and Emergency Response, Washington, D.C.

MacLeod, W. D., D. W. Brown, A. J. Friedman, D. G. Burrows, O. Maynes, R. W. Pearce, C. A. Wigren, and R. G. Bogar. 1985. Extractable Toxic Organic Compounds, Second Edition. Standard Analytical Procedures of the NOAA National Analytical Facility 1985-1986. NOAA Technical Memorandum NMFS F/NWC-92, Seattle, Washington.