SAMPLING AND ANALYSIS IN THE ARCTIC MARINE BENTHIC ENVIRONMENT

VOLUME 2 GUIDE TO PRACTICE

Prepared for:
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VOLUME 2

GUIDE TO PRACTICE

The Environmental Protection Service wishes to encourage user comments and suggestions on the content and utility of this guide. Any comments concerning the content of this report should be directed to:

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Additional copies of this report and the companion report (Volume 1; Review of Methods) can be obtained from the above address at an estimated cost of $25.00 per report.

PREFACE

This is the second part of a study to produce a guide for managers and planners involved in the collection and analysis of environmental data from marine sediments in arctic regions. The first phase of this study (Volume 1) reviewed existing methods and was intended to serve as a background document to the present report.

This report is intended to provide guidance in planning and in choosing appropriate methods for a variety of situations, conditions and objectives. The format is designed to outline in a step by step manner the factors to be considered and offers suggestions as to possible methods to achieve various results. Users are referred to other manuals or articles where detailed procedures are given.

While there is a recognized need to standardize procedures as much as possible, there is usually no concensus as to what these procedures should be. There is no single procedure that performs well under all conditions and for all parameters. Choices and compromises must therefore be made. Although this guide attempts to outline the steps in the planning process and the options available, the final choice and execution is the responsibility of individual investigators.

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1. INTRODUCTION

There is an increasing requirement for environmental information on arctic marine sediments because of natural resource development, particularly offshore hydrocarbon exploration and associated proposed transport mechanisms. At the same time, it is recognized that methods that have been used for collection, analysis and data reporting need improvement if the data are to have long term value (Thomas et al, 1982). Many of the shortcomings of past studies have been a result of a lack of awareness of the basic requirements of a sound environmental method and the limitations imposed by arctic conditions. Studies of chemical and biological sediment characteristics involve a number of steps from collection through to analysis. As in any data gathering sequence, the end result is only as valid as the weakest link in the chain of operations. In the Arctic, extreme and prolonged cold for much of the year, continuous ice cover over most regions for 8-12 months and the remoteness of the region from major population centres are all major obstacles to sample collection and analysis. As a result, sampling and field techniques have often been the weakest link in arctic marine environmental studies.

Government agencies and industries requested to provide environmental marine sediment data in support of development or to satisfy regulatory requirements are faced with a wide array of potential sampling and analytical methods from which to choose. Choosing the best or most appropriate methods can therefore be confusing and frustrating especially when there is a lack of consensus as to the best procedures and the requirements of individual situations. This guide attempts to outline the steps involved and to offer a number of choices to assist in the decision making and planning process.

1.1 Elements of an Environmental Method

The general elements of an environmental method as outlined by the ACS Committee on Environmental Improvement (Keith et al., 1983) are:

1) definition of goals;
2) preparation of a plan for sampling, analysis and reporting data;
3) sampling, subsampling and transport;
4) analysis;

5) methods of quality assurance;
6) reporting and documentation.

The goal needs to be clearly defined at the outset. This involves identifying the problem(s) and the information requirements and the use for which the data are intended. This could range from collection of preliminary information in an area where none exists to the more rigorous requirements of permit compliance or litigation. The goal needs to be clearly defined as ambiguity or too much generalization will make the goal impossible to achieve. Once the goal(s) has been established, a plan should be devised. The plan will define the population to be examined (area, extent, type of sediment, depth, species); the tests to be performed, desired performance characteristics (precision, accuracy, confidence limits); the methods for sampling, measurement and reporting given the performance requirements and intended use of the data; and finally, cost and time requirements. Definition of goals and planning are steps that must be undertaken by management or by a regulatory agency to satisfy the individual needs of different situations. The sequence of events in formulating a plan will undoubtedly be an iterative process. Goals usually require modification when faced with practical limitations. The plan must be modified if the chosen methods are not practical in terms of time, funding, logistics and facilities. After many iterations, it may become obvious that the goal cannot be achieved forcing either a modification in expectations or an increase in the allocation of resources.

1.2 Scope of the Guide

This guide deals with the development of a plan for meeting a specified objective and includes the following subjects:

- sampling strategy and means of quality control
- sampling and subsampling methods for chemical analysis
- sampling methods for benthos
- means of sample preservation and storage
- sampling logistics: transport; positioning; sampling from ice.

- methods of sample analysis for:

  metals
  organics: - TOC
  - oil and grease
  - petroleum related
  - phenols and chlorophenols
  - chlorinated hydrocarbons
  grain size
  Pb-210 dating

- methods of laboratory quality assurance and minimum requirements for reporting
- methods for the selection, collection and maintenance of organisms for bioassays
- methods for the analysis of benthic data

The following sections outline a process for selecting procedures within each of these subject areas.

2. HOW TO USE THE GUIDE

This guide is intended to aid in formulating a plan to meet specified objectives. However, users of this Guide will need to make decisions as to what methods best suit their objectives. Users must therefore provide answers to the following questions. These subjects are outside of the scope of the Guide:

- what are the goals/objectives of the study?
- when can sampling be undertaken?
- where is sampling to be undertaken?
- what level of accuracy is required or acceptable in the analyses?
- what is the time available and the approximate budget for the study?

Developing a plan will invariably be an iterative process as modifications and compromises will be required to stay within budget and allow for restrictions imposed by practical constraints in sampling and analyses. The overall plan will need to consider three areas:

1) **sampling** including where, when, how and how much to sample and sampling quality control measures;

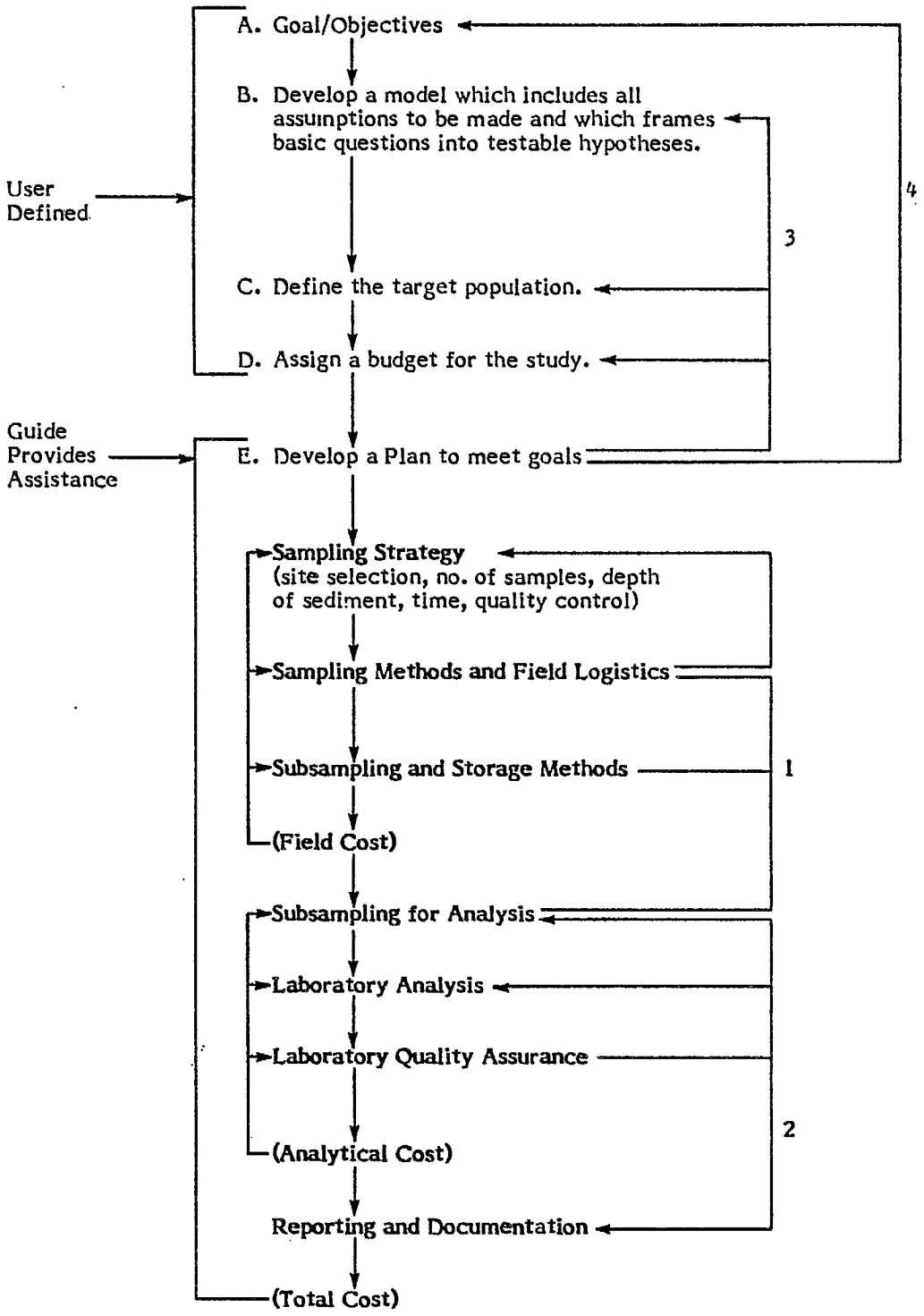
2) **analyses** including storage, preservation, digestion, analysis, and quality assurance; and

3) **reporting** of the data.

The sections of the Guide are organized in a sequence which takes the user from sampling, through analyses to reporting. The sections give a brief introduction and background. This is followed by a series of questions regarding goals and user requirements which lead to a final selection process. **Detailed procedures or descriptions of equipment and methods are not given.** Instead, users are referred to readily available references or existing manuals or guides for details.

Although sections are presented separately, it should be stressed that the final plan must encompass all aspects of sampling and analysis. It should also be pointed out that the order given in the guide need not necessarily be followed. The final plan must integrate the requirements of sampling, analysis and reporting and therefore a plan for each of these different aspects must consider the requirements and restrictions of the whole study. Definition of goals should include an assignment of priorities if there is more than a single objective. If for instance, the primary objective of a program is to obtain a certain level of sensitivity or precision in an analysis, this requirement may dictate that only one method is possible which will in turn limit the choice in other areas (e.g., the type of analytical method chosen will influence choice of sampler, sample size and means of preservation). Choosing an appropriate analytical method is often the most important step and will precede choice of sampler. If a primary objective is winter sampling, then sampling logistics and available samplers may be the critical factor and limit the types of analyses that can be undertaken. Finally, all decisions must be reconciled with the funding available. The interplay of various factors is illustrated in Figure 2.1.

A worked example is given as an Appendix for a hypothetical requirement and serves to illustrate some of the complex and often mutually exclusive requirements imposed by a broad based research or monitoring program.



Notes:
1. Choice of analytical method will dictate sampling apparatus, sub-sampling methods, sample size and storage conditions.
2. Quality Assurance Program will influence the level of reporting and documentation required.
3. Limitations imposed by components of the plan may necessitate a revision to budget, scope and/or assumptions of model.
4. If a plan cannot be devised within the constraints of B, C or D, then the goals may have to be revised.

Figure 2.1 Flow Chart of Planning Process

3. SAMPLING METHODS FOR CHEMICAL ANALYSES

3.1 Sampling Strategy

The strategy adopted for the sampling program will depend on the goal. All goals can be classed in a general sense into one of two categories:

a) descriptive: goal is to describe the present sediment or biota characteristics of an area. Examples might be a preliminary survey for a more detailed analytical plan; background survey; or simply to determine whether a particular substance is present or detectable.

b) analytical: the goal is to compare the present conditions with those of another time or area to determine effects (impacts) or changes (monitoring). Procedurally, hypotheses are formed and tested.

The type of goal (analytical or descriptive) will influence decisions made regarding where to sample (study area), how many locations to sample, how many samples should be taken at each location and how sites are to be selected. These factors are very much site and study specific. To answer these questions, users must assemble as much information as possible regarding features of the study area (ice conditions, bathymetry, currents, ice scouring, water properties and influence of river input) and the characteristics of the contaminant(s) (typical levels, susceptibility to degradation, chemical forms) and species (abundance, species richness, seasonal variations in contaminant body burdens). This information can be used to develop a model of contaminant distribution and aid in the planning process (see Section 5).

The effects of weather, ice and restricted daylight present obstacles to any arctic sampling program. These factors will dictate or influence many important aspects of sampling including: when to sample, time required, transportation, equipment performance, clothing and shelter requirements. In preparing a sampling plan, it is essential to review the available information for the region of interest regarding expected weather, usual ice conditions and transport restrictions.

There are several overviews of climatic and ice conditions which can be consulted including the Pilot of Arctic Canada, Volume 1 (Canadian Hydrographic Service); Volumes 3A and 3B of the Beaufort Sea E.I.S. (Dome Petroleum et al., 1982), and texts such as Bryson and Hare (1974) and Hare and Thomas (1974).

Detailed weather observations for stations around the Arctic can be obtained from most weather offices of the Atmospheric Environment Service, Environment Canada, throughout Canada.

The sampling plan must allow for contingencies based on actual conditions encountered in the field. Equipment malfunctions, poor weather, ice conditions, logistic problems and sediment composition can all destroy the best formulated plan. "Down" time may more than double the time required to sample especially in short surveys. The field investigator may be forced to abandon the whole attempt or, in an effort to salvage something, may make a number of sampling decisions which may or may not satisfy the goal. **Consequently a "minimum" program should always be specified, and the goals clearly established so that changes can be made that will, as much as possible, be in harmony with the stated objectives.** In the end, the success of a sampling program may depend as much on the quality of field personnel and their ability to adapt to unforseen circumstances as to the thoroughness of the original plan.

When sediments are being collected for both analysis of chemical constituents and benthic populations, the needs and constraints of both requirements need to be considered and the necessary strategies and methods integrated into the final sampling plan. Section 5 should be consulted for a discussion of hypotheses formation and testing and sampling design.

3.1.1 Definition of the Study Area

Boundaries to the study area must be established at the outset. Definition of the boundaries can be made in terms of:

- bathymetry and bottom topography (depth contours, basins, channels, etc.);
- texture of sediments;
- expected zone of influence of a contaminant input;
- practical constraints imposed by transport capability and the time and budget available;
- ice conditions

3.1.2 When to Sample

It is impossible or very difficult to sample in the fall during freeze-up and during ice break-up. Conditions are unstable and unpredictable in most arctic regions during these periods which may extend from mid-June to late July and from late September to mid-November depending on the region and local conditions. Some arctic regions will remain ice covered for a whole year. The optimum times for sampling in terms of weather and ice conditions are usually mid-August to mid-September (ice free period) and April and May (ice covered period). Most sediment surveys are carried out in the ice free season. However, when an objective is to determine contaminant body burdens in benthos, seasonal sampling should be undertaken as body burdens will vary with stages in the life cycle, health, reproductive stage and body weight of organisms. Weather records for the region and advice from local residents or persons who have worked in the area should be considered when planning the best times to sample.

3.1.3 Depth of Sediment to Sample

Depth of sediment required will be a function of the goals. However, it is **essential** in most sediment sampling for chemical analysis that the **surface layer be collected undisturbed.** In most studies, only **recent** or **present** concentrations of contaminants are important or required. Sedimentation rates throughout the Arctic will likely be 1 mm/year or less so that the top centimetre of material will represent at least 10 years of accumulation. The loss of a few millimetres of surface material therefore will mask any recent contaminant input.

Deeper sediments will be of interest in a number of situations. Dredging for instance may be to a depth of tens of metres and it may be necessary for permit applications to determine chemical constituents to the depth of expected dredging. Alternately, deeper sediments may be required or used to assess **"historical"** concentrations. This is an alternate means of providing a control in monitoring studies when the analytes are relatively stable (not easily degraded), especially when it is not practical to sample surface sediment at a control location because of time or distance constraints, or when no suitable site (i.e. same sediment characteristics but remote from zone of impact) can be found.

3.1.4 Where to Sample and How Many Samples to Collect

Choosing where to sample, how to select individual sites and how many samples to collect is perhaps the most difficult decision in the development of a sampling plan. Some guidelines are given below. However, the final decision will ultimately be a subjective one and may take many iterations to finalize. Users should also consult reviews by Kratochvil and Taylor, 1981; Green, 1979, Saila et al., 1978, Eberhardt et al, 1976.

3.1.4.1 Where to Sample

Assuming that the boundaries of the study area have been defined, it will be necessary to decide where within those boundaries to sample. Guidelines have been developed for dredging and ocean dumping related assessment. The EPA (Pequenat et al, 1981) recommends a minimum of six sites in conjunction with assessment of ocean dumping in the United States (two stations outside zone of influence - controls; two stations at the dump centre and two stations downstream or within the zone of influence). The Environmental Protection Service (EPS) in Canada should be consulted for guidance in sampling strategy in support of Ocean Dumping. In other instances, the number and location of sites must be determined on the basis of cost/site and location, goals and assumed spatial distribution of the contaminant and homogeneity of sediment within the study area. The final decision on number and location of sites must take into account the total number of samples as discussed in the next section.

The location of sites can be made on the basis of:

1) **previous site locations** in the case of a follow-up study;
2) **depositional environments.** If the goal of the study is to detect the presence of a specific constituent, then sites should be in regions of sediment accumulation. Shoals, steep slopes, sill crests and gravel beds should be excluded. **Basins and regions of fine textured sediment (mainly clay and silt) should be selected** as these are regions where contaminants will tend to accumulate;
3) **assumed or known current patterns, transport mechanisms, or deposition patterns.** For instance, Saila et al. (1978) found trace metals near a dump site in the New York Bight could be described by a log normal distribution, with metal concentrations decreasing

exponentially with distance from the dump centre. Saila et al. therefore proposed locating sample sites on a series of circles of logarithmically increasing size out from the dump centre. Positioning accuracy can be used as the criterion for determining the minimum circle diameter.

Once the potential sites have been identified it is usually necessary to choose only a portion to sample as budget and time constraints preclude sampling the whole study area in detail. There are basically two statistically defined means of selecting which sites to sample. Samples can be taken at random or in some sort of systematic way. The steps involved are:

1) The target sediment or biota is selected as outlined in Section 3.1.1 and 3.1.4.1 above. The target population (which may include the whole study area or just certain parts) is divided into imaginary units with diameters of twice the positioning accuracy or dimensions required by regulatory guidelines.

2) **For background, preliminary or descriptive surveys, or where no previous information is available, sites should be selected on a random basis.** This can be done with the aid of tables. Detailed instructions can be found in the text by Cochran (1977).

3) When the study area can be subdivided into smaller areas on the basis of physical characteristics or expected distributions (i.e. high or low probability of an impact), then a stratified system of selection can be employed. The area is first divided into strata and sites within each are selected randomly. **This is referred to as stratified random sampling and is discussed in detail in Cochran (1977).**

4) **Systematic selection of sites is a potentially more efficient means of site selection when there is background information available on the distribution of the analyte.** Systematic site selection is also applicable in determining zones of impact from a known point source of contamination. In systematic sampling, selection of the first site determines the remaining selection. Examples would be sampling at predetermined distances from a point source or in a defined spatial pattern (grid). See Cochran (1977) for details.

5) If the goal is to assess the impact of a discharge, then selection of at least one control site is important. See Section 5 for a discussion of control selection for various types of studies.

6) When currents are known, more sites should be selected in the direction of the predominant current direction.

3.1.4.2 Number of Samples

The number of samples collected will be the sum of the number of sites selected and number of samples required per site. Decisions regarding the total number of samples and how they are to be allocated among sites will be a function of:

- goals
- size of the study area
- degree of confidence required in the data and requirements of the quality assurance program
- heterogeneity of the sediment/biota distributions
- degree of segregation of the analyte in the sediment matrix
- cost (time)

Consider preliminary cost estimates: If a fixed budget is available for sampling, then an initial estimate of the cost to get a party into the field and return as well as the approximate cost of sampling per site should be made. If this is not done, then a lot of time can be wasted designing a sampling program only to find that there is time and money available to do only a fraction of it. This exercise will give a rough estimate of the time required and the number of sites that can be sampled. Section 4 (Logistics) should be consulted at this point as the logistic support required will dictate sampling expense.

When calculating the cost of sampling per site, it will be necessary to take into account the season and mode of transport. It is far more difficult (and time consuming) to sample at different sites in the winter or through ice. To move to a new site and sample in the winter or on the ice may require a whole day or as much as 5 times that required from a ship in open water. Transport requirements (to the study area and within the study area) can be costly. Ship charters can run from $ 2,000 - $10,000/day when available. Helicopter time will be in excess of $ 500/hr (see Section 4.3). These are fixed costs which must be taken into account in planning sampling expenditures.

Regardless of the goals however, certain minimum requirements can be assumed: **at least three sites should be sampled** (unless the sediments are

known to be homogeneous) and **at least two and preferably three replicate samples should be taken at each site.** Three is the minimum number required to calculate a mean and standard deviation. **In comparative studies or when plotting spatial distribution from a source, control or background site(s) should be included.**[1]

Perhaps the most important factor influencing the number of samples to be taken beyond the minimum stated is the degree of sediment heterogeneity and segregation of analyte within the sediment matrix. When no previous data are available, these factors can only be assessed by undertaking a preliminary survey or by making assumptions based on the characteristics of adjacent areas.

The variance in the concentration of a given analyte can be defined on three scales:

i) variance over the study area as a whole, s^2_a

ii) variance within the boundaries of a particular sampling site or location, s^2_L, and

iii) variance within a single sample (grab or core), s_s^2

Generally, $s^2_a > s^2_L > s_s^2$ and the number of samples would therefore be allocated in the same order i.e. number of sites sampled would exceed the number of replicates at a given site which would be greater than the number of replicates from a single sample. Taking extra subsamples from a sample entails no extra sampling costs and is usually a required quality control measure. Subsampling variance s_s^2 however does need to be known as it will contribute to the uncertainty in sampling from a site (s^2_L) and from the area as a whole (s^2_a). Some possible situations are:

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1 A means of economizing in the number of sites for some chemical parameters is to utilize a "historical" control. Deeper portions of a core sample at a site (below depth of expected bioturbation or other physical disturbances) will give a record of deposition over periods of a hundred years or more. **This type of control is only suitable, however, for analytes that do not undergo time related changes or degradation.**

If $s_a^2 \gg s_L^2$, then the emphasis in the program should be placed on sampling as many sites as possible.

If $s_a^2 \sim s_L^2$, then there should be a more or less equal allocation of samples between the number of sites and the number of replicates taken at a site.

If $s_a^2 < s_L^2$, then few sites should be sampled and the emphasis placed on obtaining more replicates at the sites chosen.

It should be stressed that the above relationships can only be used as a guide when there is sufficient background information available from previous studies or as a result of a preliminary survey. The confidence in the data will increase with increasing n (roughly as $n^{1/2}$). Analytical costs will increase roughly in direct proportion to n, while sampling costs will increase a negligible amount for extra replicates at a given site and by only a small fraction of the total for extra sites. **Therefore it is recommended that extra samples be collected.** Not all samples need be analyzed but the extra samples may help to clarify or affirm unusual results.

If the variance is known or can be estimated, the number of samples required to attain a given level of confidence in the data can be calculated (Kratochvil and Taylor, 1981):

i) where the analyte has a normal or Gaussian distribution (average greater than variance):

$$n = \frac{t^2 s^2}{R^2 \bar{X}^2}$$

where t is the student's t-table value, s^2 the variance, R the relative standard deviation acceptable in the average and $\bar{X}$ the mean.

ii) For substances or analytes whose concentrations have a Poisson distribution in the sediment (randomly distributed such that the variance is approximately equal to the average):

$$n = \frac{t^2}{R^2 \bar{X}}$$

iii) For substances with a negative binomial distribution (analyte occurs randomly in patches or clumps - variance is larger than the average)

$$n = \frac{t^2}{R^2} \left(\frac{1}{\bar{X}} + \frac{1}{K} \right)$$

where K is called the index of clumping (and must be estimated from preliminary data).

Conversely, the confidence limits for the data can be calculated afterwards on the basis of n and the observed variance and mean.

Visman's "General Sampling Theory" (see Ingamells, 1974) can also be used as a guide to choosing the number of samples to collect when sampling inhomogeneous and segregated sediment. It can also be used to assess the degree of inhomogeneity and segregation. Procedurally, two series of samples are collected, one using small sample weights, the other with large sample weights. Visman sampling constants can then be calculated on the basis of the results as:

$$A = w_1 w_2 (s_1^2 - s_2^2) / (w_2 - w_1)$$

$$B = s_2^2 - A/w_2$$

where A is a homoegeneity constant, B is a segregation constant,

s_1^2 and s_2^2 is small and large sample variance respectively and

w_1 and w_2 are the small and large sample weight.

Once A and B have been determined, the expected variance for N samples each of a weight w is given by:

$$s^2 = A/W + B/N$$

where W = Nw, the total weight of all samples.

3.1.4.3 Composite Samples

A practice sometimes carried out is to pool a number of samples, homogenize them, and then analyze subsamples of the composite. This approach will yield an average compositional value and can provide a substantial cost savings as fewer analyses will be required. Although the average of a properly prepared composite and that of a number of individual analyses will be the same, the between sample variability is lost. Since this is usually a very important statistic, **the use of composite samples is not recommended** unless it will satisfy the goals of the study. Composite samples are often a necessity however when analyzing benthic organisms for contaminants, since a minimum weight of material is required.

3.1.5 Matching the Sampling Plan to Available Funds

Once a plan has been devised, a more detailed cost estimate must be made. If the estimate exceeds the allocated funding, then obviously revisions will have to be made to the plan. One solution is to collect the number of samples as planned but to analyze only a percentage of the collected samples (i.e. cut analytical costs). The steps taken must be reconciled within the framework of the overall goals. However, many revisions to the plan may be required to finally match the sampling plan with available funds. It is wise in estimating sampling costs to include a contingency of as much as 50% of the estimate (depending on the length of the study) for delays and unforeseen circumstances, such as equipment malfunctions and bad weather and ice conditions.

3.1.6 Sampling Strategies for Detecting the Presence of Ore Concentrates or Drilling Fluids

Offshore hydrocarbon development and mining activities are currently the two main industrial activities producing wastes which are discharged directly to Canadian arctic marine areas. Drilling fluids and drill cuttings from offshore hydrocarbon exploration, primarily in the Beaufort Sea, are the major waste discharged; ore concentrates, entering coastal areas near marine loading terminals at Nanisivik N.W.T. and Little Cornwallis Island, N.W.T., have a smaller influence in terms of total amount of material discharged and the number and frequency of point sources. The sampling and analysis of marine sediment (or biota) to detect the

presence of either drilling fluids or ore concentrates requires a knowledge of the chemical characteristics of the material, size distribution of the particulates, volumes of material discharged or lost and the location of the sources. A sampling survey to detect an industrial input is an analytical exercise as comparisons and hypotheses testing would be required.

Suitable tracers of drilling fluids in marine sediments will depend in part on the carrier fluid. Water-base muds and cuttings are routinely discharged to the sea. Oil-base muds are not, at the present time, routinely used in offshore drilling operations in the Canadian Arctic. However, guidelines for their use are presently being developed.

Some potential tracers of drilling muds/cuttings are listed below:

Water-Base Muds	**Oil-Base Muds**
barium	barium
chromium (when chrome lignosulphonates are used)	synthetic organics (Biocides)
synthetic organics (biocides)	aromatics and aliphatic hydrocarbons associated with base oils

Barium is probably the single most useful tracer because (1) it is abundant in drilling fluids; (2) it usually behaves conservatively in marine sediments; and (3) there are no other significant anthropogenic inputs of Ba to arctic marine sediments.

Ore concentrate from existing coastal mining operations in the Arctic, at Nanisivik and Little Cornwallis Island, can be distinguished on the basis of metal content and particle size. Concentrates are primarily lead and zinc; the most abundant metallic impurity is cadmium (Thomas et al., 1984)

Specifically, strategies for detecting waste drilling fluids and cuttings and ore concentrates in marine sediments will involve the following:

1. **mass balance calculations and selection of suitable tracers; consideration of receiving environment concentrations and oceanographic conditions including dispersal mechanisms.**

The first step is the mass balance calculation. This involves predicting accumulation rates of contaminants in the sediments by taking into account the rate of waste discharge, physical characteristics of the waste and the concentration of significant contaminants in the discharge. The importance of this step should not be underestimated. It will provide a first approximation of the scale of the disturbance and define the detection limits required to resolve the contaminant inputs against natural (background) levels. Another benefit of having an approximate indication of the scale of disturbance is that this information can influence the frequency of sampling and the distance between sampling points.

The natural (background) concentrations of those elements or compounds which are also tracers of the waste discharges will influence the monitoring strategy by setting detection limits. As the natural concentration of a constituent decreases in the receiving environment, it becomes progressively easier to resolve the constituent associated with the discharge source from the background, assuming that detection limits are sufficiently low.

Finally, oceanographic conditions (water depth, water column density structure, currents, tides, water column particulate load, frequency and duration of high energy events, and bedform movements) indicate the likely direction of waste migration, and the rate of dispersion in both the water column and sediments. Such information will help define the requirements for sampling equipment, sample replication, sampling frequency, and station locations (offshore drilling rigs routinely collect site specific oceanographic observations).

2. **modelling the dispersion of the drilling fluids or concentrate (assumed means of dispersion, oceanographic features) to provide a framework for predicting sediment distributions.**

Saila et al. (1978) found for instance that dispersion of heavy metals from a dredge spoil dump site followed a log-normal distribution. Thomas et al. (1983) predicted an approximate log-normal dispersion of metals associated with drilling fluid disposal from offshore platforms in the Beaufort Sea. The same study predicted a bimodal distribution in Scotian Shelf sediments as a result of the increased tidal influence in dispersion.

3. **using a systematic sampling scheme to choose sampling sites with spacing based on the assumed dispersion model.** For a point source, some sort of radial plan centred on the source is probably most appropriate (Saila et al., 1978; Crippen et al., 1980) in an open sea area with no physical boundaries (see Section 5).

4. **sampling and subsampling must be designed to recover the top cm of sediment undisturbed as this will contain recent input (see Section 3.2).**

5. **if the wastes have a well defined particle size range, the sediment should be fractionated according to size and only the appropriate size range analysed to improve sensitivity.**

3.2 Choosing a Sampler for Chemical Analysis

3.2.1 Introduction

Sediment samples for chemical analysis can be collected either remotely from a vessel or through the ice, or directly by means of divers or submersibles. Remote sampling is the most common means of collecting sediment and, in most instances, the only practical one. However, direct collection using divers or submersibles, though more costly and difficult, offers distinct advantages.

There are two basic remote sampling devices which can be recommended for use in the collection of sediment samples from a surface platform: 1) grabs which take a "bite" of surface sediment; and 2) corers which collect a column of sediment.

The ideal remote sampling device for chemical analyses is one that can obtain and retrieve an undisturbed sample in sufficient quantity for all analytical requirements. The sampler should be easy to use, not contaminate the sample, have a reasonable cost and be effective for use over a wide range of sediment types. No one sampler satisfies all these requirements; sampler selection, therefore, involves a number of compromises based on the overall goals of the program, the type of sediment and whether or not depth profiles are required. The term "undisturbed" when applied to sediment sampling is a relative one. In most instances it implies that the sample has not been compacted, mixed or had loose surface material displaced. There is, however, no absolute measure of disturbance as the original sediment characteristics are not known and all samplers, of necessity, disturb the sediment

where the cutting edge or body of the sampler enters the sediment. Significant disturbance may not be visually obvious or apparent from analytical results. Subsequent data may seem plausible but be in error due to sample loss (Baxter et al., 1981). The degree of disturbance that can be tolerated will vary with the intended use of the data. If the goal is to obtain a bulk sediment, mixing of the sediment will be of little consequence. Where depth profiles are needed, however, mixing and compaction will lead to erroneous results. **Loss of the surface fines can not be tolerated if the effects of a recent input are required.**

Although there have been no definitive studies undertaken, the design features of samplers that limit sample disturbance are:

a) speed on entry: for the same sampler the degree of disturbance decreases with speed of entry.

b) surface area: the larger the open vented surface area relative to the wall thickness, the less disturbance.

c) cutting mechanism: the cleaner and faster the cutting action, the less disturbance due to mixing.

d) sampler wall thickness: thinner walls give a "cleaner" cut.

Positive contamination of sediments from samplers can arise from introduction of analyte or interfering analyte from the sampler itself, from the operation or lowering wire and from mixing of sediment from different depth horizons. The number of materials available for sampler use is limited since samplers have to be fairly robust. Almost all sampler components are metallic. As in the case of surface disturbance, there are no studies that might indicate how important sampler materials are or how severe contamination effects might be. Steps to limit sampler contamination are based on common sense and caution. Obvious features like flaking metal or paint, rust or corrosion, oil or grease, must be avoided. As a precaution, sediment for metal or organic analyses are always taken away from the sampler body. Plastic liners and core barrels are available for gravity corers and although preferable for metals, are a possible source of contamination for some hydrocarbons. Preferred materials for hydrocarbons are stainless steel and anodized aluminum. It is possible to have samplers Teflon coated, but there are no data to indicate whether this is a necessary precaution.

Sample handling is an often overlooked potential source of error. There is not the same sense of caution that is usually applied to a relatively "clean" process like water sampling. However, care must be taken that clothing, tools, lines, winches and hands do not become sources of contamination.

Beside factors which influence sample integrity, there are a number of other sampler features that are important from a user's standpoint. Obvious items are bulk and weight which will dictate lifting capacity required, ship requirements and ice hole size. More subjective factors are ease of operation and reliability. These latter aspects of sampler design are often crucial to the success of a sampling programme, especially in adverse weather conditions.

Except for very light grabs in shallow water, most remote samplers require a winch to retrieve the sampler and sediment. Lifting capacity and power requirements are important considerations. Approximate lifting requirements for various types of samplers are shown in Table 3.2.1. Also, as water becomes deeper it may be more difficult to tell when the sampler is on the bottom and a pinger may be required so that excess line is not played out which may subsequently tangle on the sampler (U.S. Naval Oceanographic Office, 1968).

Grabs and corers are different in design, and the two types of devices have very different applications. Grabs are necessary where a relatively large area (or volume) of sediment is required (i.e., for benthic sampling), and limited depth penetration is acceptable. Corers are required when depth profiles of more than about 15-20 cm are needed. Either type of sampler can be used to sample the upper 15 cm of unconsolidated soft sediments when limited quantities of sediment are required for analysis. Choice of sampler will depend on objectives, convenience and operational characteristics. Most grabs are limited by the depth of water in which they can be effectively used as their high surface area/weight ratio and shape can cause planing on descent (Bouma, 1969).

All remote sampling devices were designed for use from a vessel. Some designs require a large lifting capacity and are very bulky. Many of these devices are, therefore, not practical for through-the-ice sampling where size becomes a limiting factor.

3.2.2 Recommendations

Recommendations for sampling devices for sampling to various depths and from different platforms are given in Table 3.2.2. Further details are given below.

(a) **Grab Samplers**

Features of recommended grab samplers are given in Table 3.2.3. Of the recommended grab samplers, the Ponar is perhaps the most versatile for collecting samples for chemical analysis. It is relatively compact and yet heavy enough to be effective in a range of sediments. The Smith-McIntyre is the largest and heaviest sampler and will, therefore, be restricted primarily to shipboard use. Its frame imparts good stability however, and it is therefore, effective on a wide variety of bottoms and slopes. A modification of the Van Veen incorporating a frame and slowed entry to minimize surface disturbance has also been produced (Meek and Ray,

TABLE 3.2.1
LIFTING REQUIREMENTS FOR VARIOUS TYPES OF REMOTE SAMPLERS
(from Moore and Heath, 1978)

SAMPLER TYPE	REQUIRED LIFTING CAPACITY (kg)
Grab samplers	
Birge Ekman:	25-50
Ponar, Van-Veen	110 - 250
Smith McIntyre:	200 - 300
Gravity Corers:	
Open- barrel	200 - 2500
Phleger	70 - 100
Multiple (3 - 5 barrels)	250 - 600
Piston	200 - 2500
Box	350-400

Table 3.2.2

Recommended Samplers for Chemical Analysis

Depth of Sediments Required	Through the Ice Sampling		Open Water/Large Boat Research Vessel		Open Water/Small Boat or Aircraft on Floats	
	Type of Sampler	Comments	Type of Sampler	Comments	Type of of Sampler	Comments
a) < 15 cm	Birge Ekman	Will fit through 30 cm hole: only suitable for soft bottoms in shallow (30-50 m) water:[1]	Same as ice plus Smith-McIntyre Grab	Method of choice when a large quantity (> 500 g) of sediment required.	Same considerations as for ice sampling	
	Gravity Corer: (cylindrical; phleger, KB, benthos type). barrel I.D. 6-10 cm.	Will fit through small hole: difficult to retrieve the top few cm undisturbed: core catchers must not be used. A winch and support over the the ice hole will be required.	Craib type hydraulically damped corer	One of best samplers available for collecting undisturbed cores; capable of sampling sandy substrates: small diameter barrel; therefore limited sample: frame makes the sampler very bulky.		
	Multiple corer:	Requires a larger ice hole, but offers the advantage of collecting several cores at one time: this is extremely useful for assessing substrate homogeneity and reduces the sampling time	Box corers	Generally regarded as best samplers available for obtaining undisturbed sediment. Has a large surface area. This is the sampler of choice when a lot of sediment is required and sufficient lifting capacity available: (>500 kg): drawbacks are bulk and complexity.		
	Diving	Require large ice hole plus support equipment. Restricted to shallow (< 20m) water. Expensive: However, best method for ensuring that undisturbed sediment is collected. Allows for a great deal of flexibility in site location.				
	Compact Van Veen/Ponar	Requires larger hole than Birge Ekman: surface fines more likely to be lost: -can be used in deeper water -often used in benthos studies Van Veen sampler of choice for combined benthos/chemistry studies.				
b) >15-50 cm (approximately)	cylindrical gravity corer: e.g., Benthos, Phleger, KB, etc.). extra weights required	Will fit through small hole in the ice: surface fines may be lost. Some compaction may occur as speed of entry will have to be greater to achieve sufficient penetration: winch and some sort of support over the ice hole will be required.	As for through ice sampling plus:		Same considerations as sampling from the ice: the requirement for a winch and davit or boom may make sampling to this depth impossible from a very small boat.	
			Box corers	Comments as per (a) extra weights/lifting capacity likely required. Box corers are the best method for collecting undisturbed cores to this depth.		
	Diving	30-40 cm cores are probably the limit to which a diver operated corer can penetrate in sediment and be subsequently removed without assistance from the surface.				
c) > 50 cm	Generally not practical without drilling a large hole and without considerable lifting capacity (500 kg or more) to accommodate larger samplers. Only really practical to sample to this depth in fairly soft unconsolidated sediment.		As for ice sampling: in very soft sediments, simple gravity driven cylindrical or box corers may penetrate to 100 cm or more. If greater depth is required, a piston-type corer will be required: Sampling this depth of sediment will usually require two methods with a separate sampler being used to retrieve the upper few cm.		Not feasible unless bottom sediments extremely soft: (see comments for through the ice sampling).	

1) probably the only sampler that can be retrieved easily by hand.

TABLE 3.2.3

FEATURES OF RECOMMENDED GRAB SAMPLERS

TYPE	WEIGHT (kg)	AREA SAMPLED (cm^2)	MAX. DEPTH OF PENETRATION (cm)	SURFACE DISTURBANCE (relative)	PERFORMANCE IN VARIOUS SEDIMENT TYPES: SOFT CLAY, SILT	SAND	GRAVEL PRESENT	HARD CLAY	SUSCEPTABILITY TO SEDIMENT WASHOUT	COMMENTS	REFERENCES
Van Veen	19 - 41	- 2000	20 - 30	low-medium depending on size of screen openings	good	good	sometimes	sometimes	low, except in coarse sediment or if shells, debris present which will prevent jaws closing completely	modified Petrsen grab with long scissors-like arms which improve efficiency and penetration; short arms have been used for through-ice sampling	Mawhinney and Bisutti, Petersen, 1977
Modified Van Veen (Sontar-Van Veen)	- 80	1000	20 - 30	low					as above	mounted in a frame; sampler slides slowly down guides after frame hits the bottom; very stable; minimal disturbance; has been deployed by a helicopter in support of studies close to an offshore drilling rig	Meek and Ray, 1980
Ponar	23 - 30	400 - 550	20	low	good	good	sometimes	sometimes	low, overlapped jaws and side plates; washout may occur when gravel prevents complete closure	good all-round samplers suitable for most sediment types: jaw shape unlike van Veen, Petersen exactly follows arc of cut minimising sample displacement	Plumb, 1981 Mawhinney and Bisutti, 1981
Smith-McIntyre	45 - 90	1000	20	low	good	good	sometimes	sometimes	low, as for van Veen	basically a van Veen sampler mounted on a frame. Has trigger plates in frame so that open grab is pushed into sediment with springs (force > 35 kg); very stable because of frame; able to sample on slopes; could be deployed by a helicopter as for Souter-Van Veen	Smith-McIntyre, 1954
Birge-Ekman	5 - 10	250 - 500	10 - 20	low	good	sometimes	no	no	low, as for Petersen	poor stability in all but calm conditions, soft sediment ideal for through-ice sampling because of its compact size. Messenger triggered; spring activated jaws. Jaw shape exactly follows arc of cut minimizing sample displacement	Howmiller, 1972

1980). The main drawback of the Birge-Ekman sampler is its light weight with the result that it is ineffective on hard bottoms and is more susceptible to planing on descent. It can therefore, only be used effectively in shallow water with weak currents and relatively soft, flat bottoms. Extra weights can be added to improve performance. The Birge-Ekman is the grab sampler of choice for through ice sampling in shallow regions with soft bottoms because of its compact size and excellent operational characteristics in these conditions. **The Van Veen and Smith-McIntyre (see Section 5) are recommended for benthic studies and are therefore samplers of choice when both benthic and chemical sampling are required.**

The cost of commercially available grab samplers ranges from $1,000 to $6,000. The smaller, simpler models such as the Ponar and Ekman are in the lower price range while the Smith-McIntyre and more elaborate Van Veen modifications will be in the upper price range. A partial list of suppliers and manufacturers is given in Table 3.2.6.

(b) **Cylindrical Corers**

Features of recommended cylindrical gravity corers are summarized in Table 3.2.4. Cylindrical corer features that are desirable and should be considered in choosing a particular sampler are:

1) mode of penetration: corers that can penetrate to a sufficient depth at reduced entry speed are preferable.

2) diameter of core barrel: larger diameter cores are desirable because of the need for sufficient sediment for subsequent analyses, because of less disturbance of the core as a whole and because contamination from the corer will be less significant. Walton (1978) recommended a minimum of 6 cm I.D.. Wall thickness should be the minimum needed for the expected impact force.

3) weight and lifting requirements: corers with variable weights (i.e., weight can be added or removed) are desirable because the corer weight can be adjusted for a particular sediment or desired depth of penetration.

TABLE 3.2.4

FEATURES OF COMMON CYLINDRICAL GRAVITY CORERS

EXAMPLES	APPROXIMATE WEIGHT (kg)	DIMENSIONS	SURFACE DISTURBANCE (relative)	DESIGN FEATURES	SEDIMENT TYPES	COMMENTS	REFERENCES (See Table 3.2.6)
Phleger, Alpine	28-45	variable: core barrels usually small diameter (4 cm)	moderate	very simple design; no moving parts: uses a one-way valve system on top. Water pressure forces this up on descent. Most models have a fixed weight (e.g. Alpine): some have variable weights and stabilizer fins.	soft, sandy, semi-compacted: not a good choice for coarse-grained sediment	commercially available from several suppliers: all are steel construction with metal barrels: plastic liners and core catchers available.	Kahl Scientific Mawhinney & Bisutti, Bouma, 1969. Sly, 1969
custom-designed Phleger-type:	7 without weight. up to 7 kg per each lead weight	variable:	low-moderate (decreases as diameter of core barrel increases)	Usually aluminum frame, stainless steel cutting head; clear acrylic core barrels: variable lead ring weights; one-way valve consists of rubber bung. No stabilizer fins.	as above	easily constructed	Mawhinney & Bisutti, 1981 Sly, 1969
Benthos	up to 70	core barrel 6.6 cm: overall corer diameter ~25 cm	low-moderate	reliable design: spring activated flapper valve; stabilizer fins.	soft, semi-compacted or compacted fine-grained: not a good choice when gravel present or in sand	commercially available	Benthos Corp.
Triple Corer or multiple corer	28 (steel tubes) 21.5 (acrylic)	three (or more) core barrels: dimensions as above	low-moderate	stainless steel pipe or acrylic tubes.	clays, muds, silts	in-house modification	Kemp et al. 1971 Moore and Heath, 1978
KB™ core	8 or 18 without core barrel	5 cm barrrel: overall: ~15-20	reported to be low: depends on speed of entry	has fixed weight and a messenger-activated valve closure: that does not require water pressure to keep it open.	soft sediments	commercially available; stabilizing fin extra weight available options.	Wildlife Supply Company
Craib (hydraulically damped)	44	5.7 cm diameter core barrel	low	hydraulic damper and ball closing device at bottom for sandy sediment: corer is mounted within a frame	soft sediments as well as sand	hydraulically damped; cores are 10-15 cm long	Craib, 1965 Baxter et al., 1981

4) materials: stainless cutting heads are best; clear acrylic core barrels have the advantage that the core can be inspected after retrieval without removing it. Plastic liners are useful features as they reduce contamination for trace metals and speed up corer use.

5) stabilizer fins are useful for deep water or areas with high currents assuring that the corer will strike the bottom in a vertical position.

6) core catchers ensure a higher success rate in the retrieval of cores but can cause considerable disturbance and mixing of the sediment. **They should not be used unless a sample cannot be retrieved otherwise.** A good seal on the end of the barrel is usually sufficient to "hold" the sediment in the barrel. Valve systems that seal tightly are, therefore, desirable.

The cost of cylindrical gravity corers varies directly with complexity. The simplest gravity corers can be built or purchased for under $1,000, while a corer with a frame such as the Craib will cost in excess of $5,000.

(c) **Box Corers**

Box corers are all gravity corers with large cross-sectional area (either rectangular or square) and limited depth penetration. Because of the large sufrace area, these samplers are the best available for collecting undisturbed cores and sampling at the sediment-water interface. The large area also means a larger amount of sediment is available for a given sediment horizon while the shape of the barrel allows a side to be removed facilitating core examination and sub-sampling.

Standard boxes are fabricated from steel or anodized aluminum. Karl (1976) has developed a plastic liner which reduces potential metal contamination and speeds up sampling. Most box corers require a frame for support as the samplers are top-heavy and very unstable. All corers use some sort of shovel seal for the bottom of the box to prevent loss of sediment on retrieval. This feature allows samples to be retrieved even in very loose, unconsolidated sediment and does not affect the integrity of the core. As with grab samples, these shovels may not seal properly because of rocks, debris or shells.

The major drawbacks of box samplers are the limited depth penetration, large size and higher cost (commercial models cost between $5-10,000). The bulk of this type of sampler prevents it being of use in through-ice sampling. Features of several box corers are compared in Table 3.2.5. A partial list of suppliers and manufacturers of corers is given in Table 3.2.6.

(d) **Diving**

Diving, when possible, is perhaps the best method of collecting an undisturbed sample. However because of the much higher cost, (see Section II - 4, Volume I) diving can only be justified when combined with a benthos sampling program and when depth (<20m) and visibility permit. The advantages to diver taken samples are:

1) suitable bottom area for sample collection can be selected if a particular sediment type is required.

2) corer or grab samplers can be operated to ensure minimal disruption of the surface layer, adequate penetration into the sediment, and proper closure of the sampling device; and,

3) visual observations or photographs of sediment conditions (such as ripple marks, ice gouges, slumping, etc.) can be taken to aid in interpretation of particle size and chemical analyses.

Diver-assisted sediment sampling devices suitable for chemical studies include the Ekman and Birge-Ekman grabs, simple corers, and core tubes or boxes which can be sealed at both ends (e.g., Mawhinney and Bisutti).

3.2.3 Reliability

It should be stressed that no one sampling device will successfully collect samples from a full range of sediment types. While grabs and corers are generally reliable and robust, a back-up sampler should be included in the sampling plan in the event the primary sampler becomes damaged or fails to collect a sample because of bottom features (stones, debris). A contingency for these events should be included.

TABLE 3.2.5

FEATURES OF BOX CORERS

REFERENCE	DIMENSIONS OF BOX	WEIGHT (kg)	FRAME DIMENSIONS	BOX MATERIAL	OPERATIONAL FEATURES
Bruland, 1974	20 x 20 cm 130 cm high	350 (up to 280 kg of weights can be added)	200 cm x 100 cm	anodized aluminum	uses two overlapping shovels to seal bottom of box: spring loaded plate seal at top of the box shut on retrieval, open on descent
Bouma and Marshall (1964) Bouma, 1969	20 x 30 cm 45 cm high	$\sim$ 500 (320 kg of weight)	150 cm x 150 cm	3mm stainless steel	box open at top; single levered shovel seals bottom of box on retrieval
Texas A & M Sampler (cited in Bouma, 1969)	30 x 30 cm 91 cm high	> 600 (filled boxes weigh over 180 kg)	trapezium 120 cm on longest edge	4.7mm stainless steel	box open at top; improved versions of above sampler
Kogler, 1963 (cited in Bouma, 1969)	15 x 15 cm lengths of 1 - 4 m	can use up to 14 weights, 50 kg each	none	steel, PVC coated on interior	one way valve on top of weight stand, core nose has internal spring activated plates to prevent loss of core
Kahl Scientific Bulletin WAB-1464/1	10 x 25 cm 30 cm high	210 (with weights)	120 x 65	stainless steel	box has removeable side wall: box is sealed on top on retrieval with a flexible, thick rubber sheet

Table 3.2.6

PARTIAL LIST OF NORTH AMERICAN MANUFACTURERS OF CORERS AND GRAB SAMPLERS

1. Kahl Scientific Instrument Corp.
   P.O. Box 1166
   El Cajon, California 92022
   (all types of corers and grab samplers)

2. Benthos Inc.
   North Falmouth, Massachusetts 02556
   (gravity, piston and free-fall corers)

3. Wildlife Supply co.
   301 Cass Street
   Saginau, Michigan 48602
   (gravity corers, Eckman and Ponar grabs)

4. Hydro-Products
   11777 Sorrento Valley Road
   San Diego, California 92121
   (grabs, gravity corers)

5. TSK. Co. ltd.
   15109 - 30th Avenue. S.E.
   Bothell, Washington 98011
   (grabs, gravity corers)

6. Horizon Ecology Company
   7435 North oak Park Ave.
   Chicago, Illinois 60648
   (small grabs, gravity corers)

7. Intersea Research Corporation
   Post Office Box 2389
   La Jolla, California 92037
   (gravity corers, grabs)

8. Ocean Instruments
   5312 Banks Street
   San Diego, California 92110
   (box corers, Soutar-Van Veen grab)

3.3 Subsampling

Subsampling must be done in the context of:

i) type and number of analyses to be performed (dictates the minimum amount of sample required and the precautions involved).

ii) type of sampling device.

In terms of analyses to be performed, sections 6 - 10 should be consulted, sample requirements noted and special handling requirements and types of suitable storage containers determined.

General guidelines for sub-sampling are given below:

1) Immediately after retrieval, core barrels should be stoppered or jaws of grab samplers secured to prevent accidental loss of sample.

2) Sampler must be kept upright.

3) Surface water must be carefully removed (leaving surface fines undisturbed). If the water is extremely murky, surface disturbance will have occurred and fines must be allowed to settle, water filtered or a new core collected. This is perhaps the most critical step except when only bulk sediment properties are required (Walton, 1978).

4) Before sub-sampling, appearance of the core or grab should be noted, along with any obvious features (sediment type, colour, structures, fauna, odour). Any structures such as wood chips, large stones, etc., should be removed or not sampled.

5) For grabs where only bulk (non-depth related) analyses are required, the contents can be dumped onto a board or plastic sheet and sub-sampled according to Walton (1978). For depth related features, a small core can be taken from the interior part of the grab through the hinged tops. Sediment near the grab walls should be avoided.

6) For cylindrical cores, the complete core can either be frozen for sub-sampling in the laboratory or sub-sampled after collection. Some type of piston extruding device will be required for immediate sub-sampling (Kemp et al., 1971). Frozen cores can be thawed slightly and extruded intact then cut with a saw. Alternatively cores may be first sectioned longitudinally (Walton, 1978; Bouma, 1969), depending on the number and types of analyses.

7) Box cores can be sub-sampled by removing one side (Bouma, 1969), taking cylindrical corers in the horizontal plane throuh pre-cut cylindrical holes at set depths in the box wall or by taking small cores vertically from the surface. Sub-sampling in the horizontal plane is only recommended in very loose, high water content sediments which can be extracted easily. A large diameter cut-off syringe with the rubber plunger replaced with teflon, or metal, is a convenient minature piston coring apparatus.

8) Utensils/apparatus for sub-sampling must be of a suitable material for the type of analyses to be carried out. For trace metals, plastic scoops or core tubes should be used. For organics, metal (stainless steel, aluminum) or Teflon materials should be used. Utensils used for sub-sampling must be kept in a clean container when not in use. Some cleaning solution (solvent or dilute acid) should be available to clean utensils if they are accidently dropped or exposed to contamination (see individual sections for each analyte).

9) As much sample as possible or practical should be stored for analysis. Minimum reqiurements must be known (see Note 1).

10) The order in which sub-samples are withdrawn should be strictly adhered to and reported. Samples to be analyzed for trace constituents (metals, organics) must be taken first; samples for grain size can be taken last.

Note: 1. **Analyses are usually performed on a dry weight basis.** Water content of sediment may vary from 30 - 80%. The amount of wet sediment collected should therefore be at least 2 - 3 times the dry weight of sediment required for the number of analyses to allow for water loss and for losses during drying, grinding and sieving.

3.3.1 Storage and Preservation

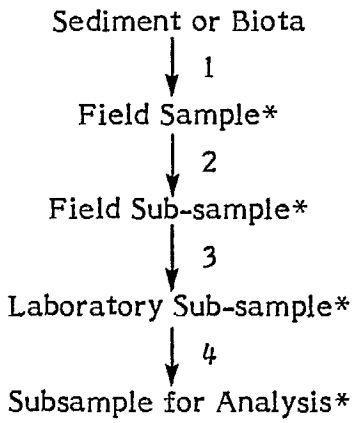
The topic of storage and preservation is discussed in Sections 6-10 for the different analytical methods. Specific requirements are given and cleaning procedures indicated. In general, the following guidelines apply for types of storage containers and preservation conditions:

TYPE OF ANALYSIS	TYPE OF STORAGE CONTAINER	STORAGE CONDITIONS
Metals:	plastic (polyethylene, polypropylene, teflon,) either bags or jars.	frozen
Organics: (all classes)	metal (stainless steel, aluminum), teflon; glass.	frozen
Grain size:	any of the above.	frozen or thawed

Note: **Sample containers must be robust:** glass containers need special packaging to prevent breakage in transport and must not be overfilled to prevent breakage during freezing. Most plastic containers become brittle at very low temperatures (-30°C or less) and will shatter or break if dropped.

3.4 Quality Control in Sampling

The steps involved in the sampling and subsequent reduction of a sample to a laboratory size suitable for analysis are indicated below.



Each of the above steps is a potential source of error. Steps 1 and 2 are carried out in the field and how well they are carried out is crucial to the reliability of the subsequent data. Procedures need to be incorporated into the sampling plan to provide quality control and to assess the magnitude of the error in the sampling and sub-sampling procedures. The topic of quality control in the laboratory is discussed in section 10. The goal of quality control measures in sampling are to control sources of error and assess the sampling and storage variability. It is not possible to measure the accuracy of the sampling as the "true" value and composition of a sediment are never known beforehand. Instead, measures of precision obtained by examining distributions generated if a procedure is applied repeatedly to the same population are used. Some elements of quality assurance applicable to sampling are listed below.

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*Note: There is an important distinction between a "sample" and a "sub-sample" (Ingamells, 1974) A single sample of sediment will rarely if ever be representative of the sediment or biota as a whole. The composition of the sediment can only be determined by taking a very large number of samples (i.e., step 1 must be done repeatedly). A sub-sample however, is usually considered to be a representative split or portion of a sample (sub-samples from the same sample should have the same composition). This distinction is important when considering quality control measures and in designing a sampling plan. The most replication should be concentrated in Step 1 (see Section 3.1.3.2).

1) **Replicate Samples:** At randomly selected sites, more than one sample is collected (includes variability due to sampling equipment, subsampling procedures and substrate heterogeneity).

2) **Split Samples:** From a single collected sample, two or more subsamples are taken and stored (includes variability due to subsampling procedures and heterogeneity within a single collected sample).

3) **Collection of two series of sub-samples each of a different weight** (size). The results of analyses from each series of weight can be used to calculate Visman sampling constants for homogeneity and analyte segregation within the sediment and the expected subsampling variance (Ingamells, 1974).

4) **Field Control Samples:** Uncontaminated sediments are spiked with known amounts of the analyte(s) of interest.

5) **Preservative Reagent Blanks:** If a preservative is used, its quality should be routinely checked - see section 10.

6) **Control Site Selection:** When an objective of the program is identification of elevated levels of a particular analyte (contamination), a site or sites are usually selected that are thought to be uncontaminated for comparative purposes. Proper selection of control sites is important if data are to be correctly interpreted (sediment characteristics need to be closely matched). This is part of site selection (section 3.1.2).

7) **Selection of Proper Samplers:** Samplers must be selected which can retrieve the desired type of sediment, are non-contaminating and can collect a minimally disturbed sample. (See section 3.2)

8) **Selection of proper storage containers and storage conditions.**

Field control samples and replicate sample results must be treated with some caution. In field control samples, the added analyte may behave and be extracted differently than naturally occurring analyte. Quantitative recovery of the field spike does not mean naturally occurring analyte will be quantitatively recovered or behave in the same way. Under recovery however, will indicate a problem. Results for replicate samples will include variability due to the sampler, sample handling, storage, as well as the heterogeneity of the substrate. In some areas, the latter variability may be much greater than all other factors. Only replicate samples taken from a known homogeneous sediment can be used to estimate sampler and sampling technique uncertainty (precision).

3.5 Requirements for Legal Samples

Whenever it is known or suspected that samples will be required for litigation, additional precautions are required in sampling, handling, storage, transport and analytical procedures.

Dick and Pant (1978) summarized three fundamental objectives when dealing with legal samples for oil spills which can also be applied to sediment. These are:

1) the preservation of sample integrity so that the sample composition is the same as at the time of sampling;

2) the proof of continuity of the sample from sampling through to final documentation (chain of custody); and

3) the use of analytical techniques capable of providing positive identification of the pollutant.

Maintaining sample integrity is a function of the sampling and storage procedures and is the goal of all sampling programs. Available analytical techniques are discussed in Sections 6-10. The proof of continuity of the sample is to assure and document that the sample(s) is in the physical possession of a specified individual or in a secured place and could not have been damaged or altered (Katz and Jenniss, 1983). This requires special procedures not usually followed in routine applications. There are apparently no well defined rules to ensure continuity. Dick and Pant (1978)

in their review outlined various methods in current use in Canada and the United States. The recommendations regarding sampling and sample handling are summarized below:

3.5.1 Sampling and Sample Handling

In preparation for field sampling, a sufficient number of appropriate containers should be assembled, with etched or permanently marked identifying numbers on the containers as well as on matching lids. The containers should then be properly cleaned and prepared, as specified for the particular analyte, by one lab person only. To reduce the possibility of contamination, the sample containers, lids and labels should be sealed individually into polyethylene bags. The containers are then packed into a locking sample box in readiness for field sampling. The box is marked "legal sample bottles", accompanied by the date and a signature of the person who prepared them, and then locked. At this point, the sample box key is given to the person in charge of sampling and the sample box is transferred by this person to the sampling site.

At the sampling site, field personnel are required to keep a field notebook of all pertinent information. For each sample taken the container number, date sample taken, time sample taken, source of sample (with reference to an area diagram), preservative, if used, and physical characteristics, where applicable, should be recorded in the field notebook as well as on the label supplied with each sample container. The field sampler's name and signature, as well as that of an emergency or investigating officer or witness should be recorded on each sample label (if possible). The label should then be securely fastened to the sample container and taped over with transparent tape to preserve the information. The container number is cross checked in the field notebook with the information on the label.

After being filled with the sample, each container is sealed and should be cross-taped and marked in such a manner as to prevent contamination and leakage, and to indicate if the container has been tampered with.

At least two sample containers should be left empty as a control, to prove that the containers were not contaminated before sampling. All possible suspected sources relative to each sampling site should be sampled, with a minimum of two samples from each location.

The sample box, with all the sample containers, is transported with the crew to each sampling location. Samples and the sample box itself, are not left

unattended. After completion of sampling, the sample box is locked. The key should be kept on the field investigator's person at all times, and the sample box is clearly marked as to its contents. Samples must be in the field investigator's actual physical possession; or in an area where they can be kept under surveillance by an authorized person; or in a limited-key-access locked refrigerator or storage area, where they cannot be tampered with until such time that they can be brought to or sent to the appropriate laboratory for analysis.

Samples can be sent to the lab for analysis by registered mail, air freight or air express. Any transport documents associated with shipping, such as a hazardous material shipping form, are retained for the legal file. All wrappings and sample containing boxes are all similarly identified. A covering letter is prepared with similar identification and one copy is sent separately to the lab involved while another copy accompanies the samples. A memorandum describing the samples and the type of analysis required is included in the sample box. A key to the sample box is sent in a separate envelope and not attached to the box. The box lock is secured and taped-over. An added precaution that has been suggested is a photograph showing the condition of the box.

Hand delivery of the samples to the laboratory by the field personnel is preferred. The associated identifying documentation, memorandum of sample description and required analysis is also hand delivered. The laboratory person receiving the delivery and the person making the delivery sign a transmittal slip with copies being kept by both parties. The name of the person making the delivery is noted in the case file. A note of the transmittal is recorded in the laboratory record book or file and any additional receiving data is then noted, such as evidence of tampering. All packaging and wrappings are dated and initialled by the analyst and then retained on file.

Deliveries by registered mail or courier should be received by the analyst assigned the task of carrying out the analyses for the samples in question. Upon receiving the sample box, the analyst should take a photograph of it, to compare with a photo taken in the field. All registration slips or waybills should be kept with the box, and any wrappings initialled and retained.

With the arrival of the samples in the lab, a case file should be opened and case numbers assigned to each sample. A continuity sheet is prepared and the samples examined in conjunction with the memorandum requesting analysis.

All samples are marked by the analyst with his/her initials, the case number, the sample number and the date of receipt. All memoranda and associated documents are similarly marked, and the condition of the samples noted in the laboratory notebook.

One analyst should carry out all of the analyses and keep all samples, process solutions, etc., under lock and limited access key when not under direct surveillance. Notes are taken of all aspects of the analysis. All such notes, graphs, charts, and rough drafts of final reports should also be kept under lock and limited access key during the analytical process and when not in direct use.

Subsequent to laboratory analysis, the analyst should ensure retention of sufficient post-analysis sample for defence or rechecking purposes. Where adequate storage facilities exist, the post-analysis samples are retained under lock and limited-key-access until prosecution is completed or until the case is dropped.

If adequate locking storage space is not available at the lab or cannot be arranged, the analyst should return the samples with the final report to the originating field group. The samples are transferred either by hand, registered mail, air express, air freight or courier. If transferred by hand, a signature is obtained on the laboratory continuity sheet from the person receiving the shipment. If shipped by registered mail, express, etc., all documents involved in making the shipment and transmittal are retained in the case file. The receiver of the shipment signs the continuity sheet and holds the samples in a locked, limited access key area until the legal process is completed or the case dropped.

4. SAMPLING LOGISTICS

Implementing a sampling plan in the Arctic requires considerable logistic support and expense. Practical considerations must be taken into account and these will influence choice of samplers, sampling strategy and types of analyses. The factors to be considered in this section are:

- positioning
- transport
- sampling from the ice
- sampling from ships
- personnel requirements

4.1 Positioning

The required accuracy and precision of station positions will depend on the type of study and the size of the study area. Besides the performance capability, the other important considerations in the choice of a positioning method are:

- **range:** it is preferable to have one system that will cover the entire study area.
- **size:** the size and complexity of positioning equipment are important for transport.
- **cost:** this factor is obviously a major factor.

Recommended positioning systems are summarized for a variety of applications in Table 4.1. A brief description of other positioning systems is given in volume 1, section II-6.

4.2 Transport

Sample transport from arctic regions is the portion of the analytical chain linking the field and laboratory portions of the study. This is a critical step, as the

TABLE 4.1

RECOMMENDED POSITIONING SYSTEMS FOR VARIOUS APPLICATIONS

SAMPLING PLATFORM	SURVEY TYPE	ACCURACY REQUIRED	APPROXIMATE COST[4]	SUGGESTED SYSTEMS
1. Shipboard	Wide area survey	100-500 m	$2,000 - 5,000	SATNAV[3](offshore) SATNAV[3] plus RADAR (nearshore)
		1-10 m	$50,000	ARGO, SYLEDIS
	Nearshore	10-100 m	>$5,000	RADAR[1]
		1-10 m		MINIRANGER/TRISPONDER MICROFIX
2. Small Launch	Nearshore	10-100 m	<$2,000	SEXTANT,PELORUS[1,2]
		1-10 m	$5,000+	MINIRANGER/MICROFIX/ TRISPONDER
3. Ice	Wide area survey	100-500m	$2,000 - $5,000	SATNAV
	Small area	10-100 m	<$2,000	SEXTANT, PELORUS THEODOLITE[1,2]
	Small area	1-10 m	>$5,000	MINIRANGER/MICROFIX/ TRISPONDER Lines along ice form fixed point.

1. suitable targets required
2. Good visibility required.
3. Continuous positions not available; dead reckoning required between fixes.
4. Purchase price: most systems can be leased or rented.

investigator loses control of the samples. Precautions must be taken to ensure the integrity of samples during transit and their timely delivery to the laboratory.

In the Arctic, all transport will ultimately be by air, perhaps with several transfers. The cost of air and ground transport can be a large component of the overall sampling expenditure. Charter fixed wing aircraft generally cost from $2.50 - $5.00/mile plus fuel. Helicopters cost from $500/hr plus fuel for a small 4 seater to over $1,000/hr plus fuel for larger machines. Prior to the trip, air carriers, routing, schedules and cargo restrictions must be known so that transport can be planned ahead. Few northern coastal communities have daily flights to southern Canada and all will have weight/size restrictions for cargo. A list of air carriers, licence restrictions and addresses for major arctic communities is given in Appendix C of volume 1. Some points to be considered when planning sample transport are:

1) cargo capacity of the aircraft (limiting dimensions for a single item and weight restrictions);

2) schedules: general availability of space, rates, priority freight service;

3) terminal facilities: are there facilities to keep samples cool, frozen, or to keep them from freezing in winter at transfer points?

4) restricted items: several items such as dry ice, concentrated acids, formalin, batteries (wet), gasoline cans, gasoline motors may not be accepted for carriage on passenger carrying aircraft. All restricted items require proper documentation regardless of aircraft and packaging. Failure to do this can cause delays or result in loss of necessary items (as well as potentially severe penalties). IATA (International Air Transport Association) publishes a listing of all restricted items, along with instructions for proper packaging, labelling and documentation. Permissable quantities are also indicated. The most recent IATA regulations should be consulted when planning a field trip (may be obtained from IATA, Traffic Services, 2000 Peel Street, Montreal, Quebec, H3A 2R4);

5) packaging: wooden, metal or hard plastic should be used when possible; some plastics become very brittle at low temperatures. All plastic containers should be tested before being used to transport materials in winter months. Cardboard boxes should not be used;

6) labelling: all items to be shipped need a number, the contents of each box should be recorded; labels should clearly show destination, special handling instructions and phone numbers to call on arrival; restricted items need proper documentation (item 4);

7) cost: charter rates, freight rates can be a major factor in the overall budget. In planning a sampling program, allowance should be made for the extra weight of samples on return trips;

8) timing: depending on the amount of equipment to be transported, it may be necessary to ship equipment ahead. When personnel follow, they can then ensure that all equipment reaches its destination on time.

The key to successful transport is good planning as well as the necessary flexibility to deal with forced delays due to weather.

4.3 Sampling from the Ice

Sampling from the ice is practical only when the ice is relatively stable. In nearshore areas this means when the ice has achieved sufficient thickness, usually a month or so after freeze-up until a few weeks (or less) prior to break-up. Further offshore and in many coastal regions multi-year ice may be present all year. Sampling from the ice has some advantages, most notably a stable platform and large surface area, as well as some obstacles not encountered in more traditional ship-board sampling, including access to the water, shelter and transport.

There are no standard procedures or equipment for sampling from the ice. Requirements and equipment vary with the application, transport restrictions, time of year, personal preferences and available resources. There are, however, several factors that should be considered when planning, namely:

1. site selection;
2. drilling holes in the ice;
3. positioning;
4. shelter and safety requirements;
5. transport;
6. equipment, power, winch and retrieval requirements; and
7. a practical sampling device.

The factors in sampler selection will be a function of goals, parameters to be analysed and portability. These topics have been reviewed in Sections 3.2 and 5. There is a practical limit to the size of hole that can be readily made in the ice. In general, the most compact sampler (smallest cross-sectional dimensions) capable of satisfying the objectives should be used. The other factors are discussed briefly below.

4.3.1 Site Selection

In first year, land fast ice, site location is usually not a problem. The ice is relatively flat and a uniform thickness. In transition zones or multi-year ice however, site selection is more difficult. This ice can be very uneven and broken. An area of flat first year ice is preferred as this will have a minimum thickness and provide a flat landing area for aircraft and equipment. Where the ice is ridged or tilted, ice thickness may be much greater than anticipated. This will require more time to make a hole in the ice or perhaps make it impossible with the equipment available. Multi-year ice will be thicker (2 - 10m) and harder in general. **Sampling on transition or multi-year ice will require considerable flexibility in site location. The number of sites and their location will be dictated by local conditions and cannot be planned in detail in advance (Section 3.1).**

4.3.2 Making a Hole in the Ice.

Once a location has been selected, a hole of sufficient size for the sampling gear is required. Small corers without stabilizer fins may require a hole of 25 cm diameter or less. Small grab samplers such as the Ekman will require a hole at least 30 cm in diameter while larger samplers like the Van Veen (short armed version) will

require a hole for deployment at least 80 cm in diameter. If a small diameter auger is available, it may be advantageous to drill a small diameter hole first to determine ice thickness. This can save a lot of time if the ice proves to be too thick for the equipment available.

There are a number of ways of making a hole in the ice, depending on the ice thickness, size of hole required and the equipment available. In early winter or when the ice is relatively thin (30 cm or less) a sharp ice chisel is the simplest means available. The most common method however is to drill a hole using commercially available ice augers and power drives. The augers can be driven by gasoline powered engines, by electric motors, by a hydraulic drive or by hand. It is generally not practical to use a hand auger for holes greater than about 10 - 15 cm in diameter, which is too small even for gravity corers. With a power unit, either gasoline driven or electric, up to 45 cm holes can be drilled, though larger sizes (> 25 cm) require additional horsepower in the drive unit and are usually heavy. These units can penetrate 2 m of ice in about 10-20 minutes. If a large track vehicle is available, a vehicle mounted drill can be used driven by vehicle power or hydraulics and larger diameter augers used (up to 60 cm).

Portable gasoline powered engine drives have the advantage of portability, wide spread commercial availability, and low cost (typically under $1,000). The units are heavy however, and can be difficult to start in very cold weather. Two people will be required to drill smaller diameter holes (to 25 cm), with an extra person required for larger diameter units in thick ice.

Electric drills are also readily available. However, they require a power source (generator) which may present problems. Large drills with up to 1 hp motors are required.

For large size holes, chain saws with long extensions have been used. This is a laborious, time consuming (usually several hours) and messy procedure which is not recommended for routine work. However, for a limited number of sampling sites this is an effective means of making a large hole. For diving this may be the only practical method for making a large enough hole.

Perhaps the best and certainly most versatile method available is the hot water drill. Hot water drills are not available commercially but can be built from readily available components and materials. Verrall and Baade (1982) developed a unit for drilling holes in ice shelves along Ellesmere Island. They were able to put a 23 cm diameter hole through 50 m of shelf ice at a rate of 11 m/hour.

The Frozen Sea Research Group (FSRG) at the Institute of Ocean Sciences, Pat Bay, B.C., have also developed a hot water drill for use in arctic applications. The FSRG unit works on the same principle except that they utilize an oil burner (3 USGPH fuel consumption) and have produced a helicopter and Twin-Otter fixed wing aircraft portable design. FSRG also utilizes a melting ring rather than a single jet outlet. Two perforated rings are used in tandem, one for the outlet of hot water and one for collecting cooled return water. This system allows a great deal of flexibility as rings of various diameters can be easily fabricated depending on the application. Very large holes can be made by drilling several holes side by side. Using a 91.5 cm diameter ring, the FSRG unit can penetrate 2 m of ice in about 35 minutes. The ice block that is produced can either be pushed down under the ice or removed. The ice hole produced by either method has the additional advantage of being free of slush and ice chips produced in other drilling methods.

The chief disadvantages of this method are the requirements for a generator to run the pump and heater and the lack of a commercially available system.

4.3.3 Shelter and Safety

Except for ideal conditions in late spring or early summer when air temperatures may be above freezing, some sort of shelter will be required while on the ice. The type of shelter required will depend on the length of time spent on location, weather conditions, transport limitations and sampling gear. The simplest shelter is a windbreak but usually a heated area is needed both for personnel and equipment. This may be the transport vehicle (all terrain vehicle, snow-trak, airplane or helicopter); a portable insulated shelter or tent (Parcoll, Hansen or Norseman tent); a trailer that is either helicopter portable or mounted on skis; or a snow hut/igloo. Depending on clearance requirements and size of ice hole and sampler, sampling may be carried out within the shelter.

Sampling on the ice demands that the sampling party must be totally self-sufficient. Equipment requirements should reflect this and allow for emergency situations (communication equipment, survival gear, food, fuel, first aid equipment). Sampling on the ice also requires that the sampling party be aware of the dangers of an encounter with polar bears should the party be on the ice for a long time. Some sort of watch should be maintained and a rifle of suitable power (as well as someone with the knowledge of how to use it) must be readily available. Information on polar bear deterence strategies may be obtained from the Government of the Northwest Territories, Department of Renewable Resources, Habitat Management in Yellowknife.

4.3.4 Transport

Depending on the distance to be travelled and the amount of equipment required, a number of possible means of getting to a location are available. For short distances close to a community or camp, a snow vehicle (Snowmobiles, Bombardier, ATV or Cat) with a sled for equipment is suitable. Snowmobiles and sleds can be rented in any northern community. As distances increase, all transport will eventually rely on aircraft, either helicopters or fixed wing. Helicopters are more versatile but also more expensive to charter and not as readily available. Choice will depend on the area to be sampled, amount of equipment and distance to be travelled. Fixed wing aircraft are required when distances are great (generally more than 100 km). Helicopters have advantages on shorter flights in terms of flexibility (landing), in site selection and in handling bulky items (these can be carried in a sling under the aircraft). People cannot be transported simultaneously when equipment is carried in a sling. Aircraft cannot be used when visibility is poor, as a result of weather conditions, or darkness, so that long delays may result at certain times of the year.

4.3.5 Power, Winch and Lifting Requirements

With the exception of the small Birge-Ekman dredge, almost all sampling will require a winch and davit (or boom) to lower and retrieve the sampler (see Table 4.2). Lifting requirements and the size of winch and power required as much as the sampler dimensions will dictate the type of sampler that can be used. All equipment must also be portable enough for aircraft transport. Winches can be gasoline powered, electric or hydraulic. Drive units for ice augers can often be adapted to drive a winch. Tripods or quadrapods have been used to support the block above the ice hole. Winches can be supported directly on the frame.

There is no commercially available equipment "ready to go". Equipment components must be designed and mated to satisfy the sampling needs. Experience from others who have sampled from the ice will be helpful. Ultimately, however, investigators must adopt methods to suit their specific requirements and must thoroughly test out equipment and procedures before finalizing a sampling plan.

4.4 Sampling From Vessels

Vessel requirements for sediment sampling in arctic waters will be the same as for other marine areas with the exception that, in some instances, vessels with an ice-strengthened hull will be required. **A major constraint to programs requiring a large scientific vessel is the general lack of suitable vessels in the Arctic.** Choices will be very limited and costs will be higher. Charter costs for a 40' fishing vessel with operator when available will be in the range of $2,000/day plus fuel. Larger vessels must usually be brought in from southern areas at great expense. In more remote areas, the only boats available may be 15' - 20' fishing boats or canoes, or inflatable boats flown in. This will obviously restrict the type of samplers that can be used and the sampling plan (section 3).

The following factors should be considered when formulating a sampling plan for sampling from a boat:

1) lifting capability: winches, line (strength, material and length), blocks, booms, cranes, A-Frames, davits; (see Section 2.3 for approximate lifting requirements; see U.S. Naval Oceanographic Office, 1968; Stirn, 1981, or Holme & McIntyre (1984) for details). Table 4.2 outlines typical applications for various types of winches and wire rope;
2) clearance over the railing with available lift capacity (function of sampler dimensions);
3) open work area for sampler manipulations, storage, etc.[a] (this will depend on the type of sampler, the amount of equipment and number of people required to launch and recover sampling gear);
4) laboratory space (for sub-sampling, sample work-up);[a]
5) storage facility (freezer and refrigerated space);
6) navigation equipment (see Section 4.1);
7) accommodation;
8) range (fuel capacity, rate of consumption, speed);
9) anchoring capability;
10) availability of electrical power;
11) availability of running water (benthic sampling);
12) overall dimensions, sea-worthiness, ice rating; and
13) communications - types of radios, frequencies available and range.

[a]Note: Ships present very dirty environments for sample handling. Special care must be taken to prevent contamination.

TABLE 4.2

TYPICAL APPLICATIONS FOR VARIOUS WINCHES AND WIRE ROPE LINES

DUTY	APPLICATION	WIRE ROPE SIZE (mm)	LENGTH OF LINE (m)	LOAD (kg)	SPEED (m/min)	MOTOR (H.P.)
Light	smallest grabs	2.4 - 3.2	400 - 800	50	300	3 - 5
Medium	all grabs, light to medium weight cylindrical and box corers	4 - 5	up to 5000	250 - 500	100 - 300	15 - 20
Heavy	piston corers, large box corers and gravity corers	9.5 - 12.5	to 12,000	14,000 - 3,600	40 - 120	30 - 150

4.5 Personnel Requirements

An important part of any study plan is the allocation of personnel. Given the uncertainties in field programs, the necessity for compromise and adaptations, the quality of field personnel may be considered one of the most important factors in a successful sampling program. Both the number of people and their level of training and experience are important considerations. There are no set rules for estimating personnel requirements, although certain tasks require a minimum number of people regardless of training.

Table 4.3 lists guidelines for establishing personnel requirements for various types of sampling apparatus. However, if sample processing or work-up is required while sampling is taking place, then additional personnel will be required. **At least one member of the sampling team must be experienced in all the techniques to be used.**

Table 4.3

Personnel Requirements: Sampling Methods

Type of Sampler	Relative Difficulty	Number of People Required to Deploy and Retrieve
A. Grabs		
Small grabs (e.g. Ekman, Ponar)	simple; requires little training or technical skill: easy to handle because of small size as long as deployed in shallow water	1 (possibly 2)
large grabs (Van Veen)	simple; requires little training to successfully deploy; heavier	usually 2 (and winch)
grabs with frames (Smith-McIntyre)	most complicated of grabs to operate; heavier and more bulky; however, these grabs can be used with minimal training	2 (and winch)
B. Corers		
cylindrical gravity	easy to use; little training required	2 (and winch)
box corer	most complicated; requires an experienced person to operate successfully: training in field almost essential	2 - 4 (and winch)
piston	complicated, potentially dangerous: awkward to handle; should be handled by experienced personnel only	2 - 4 (and winch)
damped, framed (Craig)	complicated; requires previous training	2 (and winch)
C. Diving	must be experienced divers	minimum of 2

Note: The most demanding part of sampling is being able to recognize problems (i.e., disturbed sediment; excessive washing) and being able to adapt equipment to changing conditions. This cannot be taught - actual field experience is the most important factor. Although no specialized educational background is required some technical competence and a thorough understanding of the principals of how the sampler works are required.

Subsampling requires special care: the person subsampling must be familar with the study goals; sources of contamination and how this can be avoided. A trace metal or trace organic chemist is not necessarily required. However, very explicit instructions need to be provided if a chemist is not available. The instructions should be clearly written out by the study scientist in conjunction with the chemist responsible for the analyses.

Field processing (sieving) of sediment for benthos requires someone with a basic knowledge of benthic organisms. Explicit instructions need to be provided if a benthic biologist is not available to carry out preliminary processing.

5. METHODS FOR THE STUDY OF MARINE MACROBENTHOS

5.1 Introduction

The objective of this section is to outline the basic steps in planning and carrying out applied environmental studies on marine macrobenthos. The approach taken here is to first indicate major elements for planning applied environmental studies. These include (a) the conceptual steps in planning and executing a study and (b) basic considerations of sampling design and evaluation. Selection of suitable benthic sampling devices is included as a facet of sampling design. Next, the five major types of applied biological studies are described along with a framework for deciding which might be applicable to a particular sampling problem. For each main category, the basic options for sampling design, statistical analysis and sampling allocation are outlined.

Finally, the procedures for sampling macrobenthos and methods for treatment of benthos samples are considered.

Excellent general references on sampling design and statistical methods for applied biological studies are Elliott (1977), Green (1979), and Allan (1984). If the user is not familiar with statistical tests it is recommended that the advice of a statistician be obtained at the planning and design stage rather than after the samples are collected and analysed.

5.2 Elements of Planning for an Applied Study of Marine Benthos

The structure of an environmental study should be based on the logical progression from objective → question → hypothesis → sampling → design → statistical analysis → tests of hypotheses → interpretation and reporting of results (Figure 5.1). The biologically defined objective must preside over and use the statistical methods instead of the opposite (Skellam, 1969; Green, 1979).

5.2.1 Hypothesis Formation

The first step is to form hypotheses from the questions arising from the objective of the study. The framing of a testable and falsifiable null hypothesis (H_0) is necessary before a statistical test can be applied. This means that the results

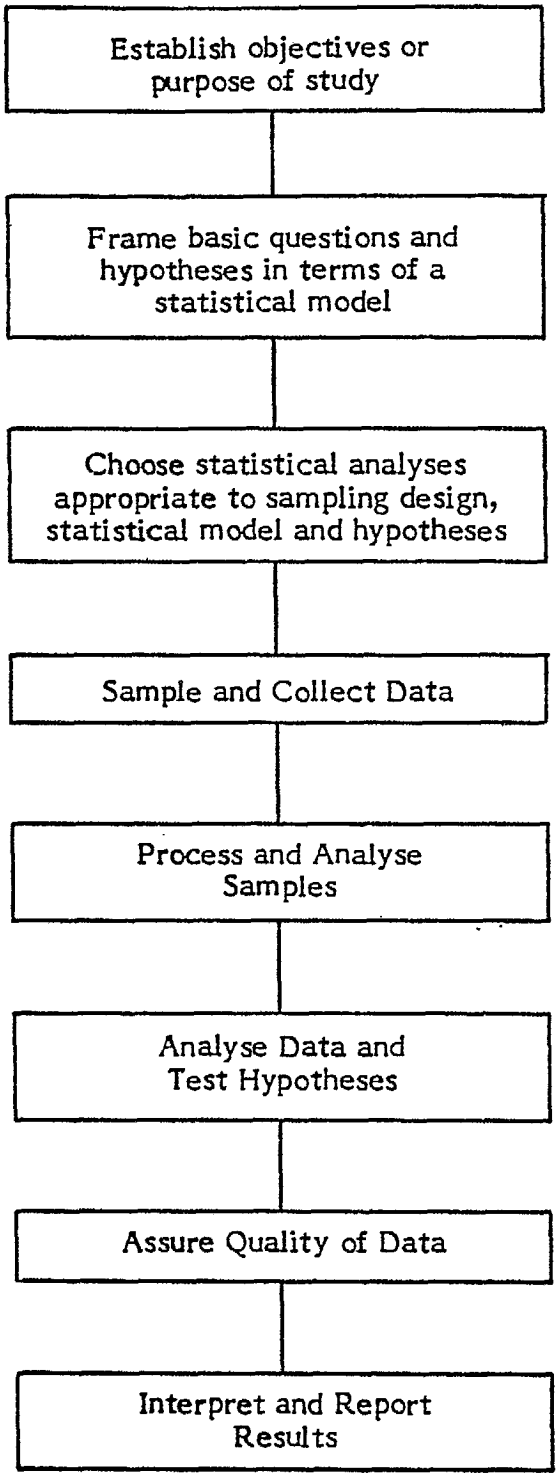


Figure 5.1 Conceptual Steps in Planning and Executing an Environmental Study.

cannot be explained so that they support a given hypothesis regardless of what the results are. There must be possible results that require each of the two possible decisions: accept H_o or reject H_o. The null hypothesis proposed should be the simplest possible answer to the question arising from the purpose of the study. Sampling and statistical analyses follow from this.

When statistical analysis is applied for exploratory descriptive purposes, tests of H_o: "nothing is going on" may be appropriate. For example, multivariate methods such as cluster analysis and ordination are often applied to reduce large, complex data sets to "what is going on". Because these procedures can give results that have the appearance of something going on even when used on random simulated data, it is advised that the H_o: "nothing is going on" be rejected before proceeding with this type of descriptive analysis (Williams and Lance, 1965; Goodall, 1966; Green, 1979).

5.2.2 Statistical Models

A proper null hypothesis is generally expressed in terms of a statistical model which states some hypothesized functional relationship between one or more dependent variables and one or more independent variables. For example, the dependent or criterion variables could be measures of biological impact effects. The independent or predictor variables could be measures of impact intensity or also of environmental variation which may contribute to variation in the criterion variables even though it is not related to the impact itself. The perfect statistical model would have the properties of generality, realism and precision (Levins, 1966). In practice, one of these must be jeopardised, which determines the strategy to be taken. For instance, generality may be lost if one is concerned with predicting what will happen at a specific location. In another case, a high degree of precision may not be needed if a biological response has a large amplitude. It is important, to give considerable thought to the choice of an appropriate statistical model because this can be a more serious source of error and deception than errors in estimation or hypothesis testing (Skellam, 1969; Green, 1979).

5.2.3 Sampling Design and Evaluation

The sampling design should be as compatible as possible with the statistical model of the null hypothesis (Steel and Torrie, 1960; Elliott, 1977; Green, 1979).

Criterion and predictor variables must be chosen carefully to ensure they really measure what they are intended to. Therefore, the choice of an appropriate sampling device and an understanding of its limitations in sampling the target population are important facets of sampling design (Figure 5.2). Factors to consider in the choice of a suitable sampling device for benthos are:

1) nature of the substrate (hard or soft)
2) target population (infauna or epibenthos)
3) desirability of direct observation of benthos and habitat
4) depth of study area (<20 m or deeper)
5) sampling platform (vessel in open water, or from ice)
6) cost

A flowchart of decisions and possible solutions is given in Figure 5.3. Characteristics, advantages, disadvantages and approximate costs of various types of sampling equipment for macrobenthos are provided in Table 5.1. General references on sampling gear for benthos are Holme and McIntyre (1971, 1984) and Holme (1964). A review provided in Sections 4 and 5 of Volume I considers various types of remote and direct samplers for macrobenthos.

A highly desirable feature of sampling design is that sampling be truly random at some level. If not, the assumption of independence of errors may be violated, making most statistical tests invalid. This condition is impossible to remedy after the data have been collected, but can be avoided by truly random allocation of samples. Further discussion of this topic is presented later in relation to error variation.

Sampling design should aim to control or hold constant as many as possible of the environmental variables which may be correlated with the biological criterion variable(s). For example, in the design of an impact study, control or reference areas and impact areas should be selected so that they have similar environmental conditions except for the projected presence or absence of the future impact.

Equal numbers of randomly allocated replicate samples should be taken within each combination of location, time, and any other controlled variable. For most statistical tests of hypotheses, an estimate of the amount of variation within as well as variation among each combination is needed. Estimation of variation within requires replication. A good example of an environmental study with consistent,

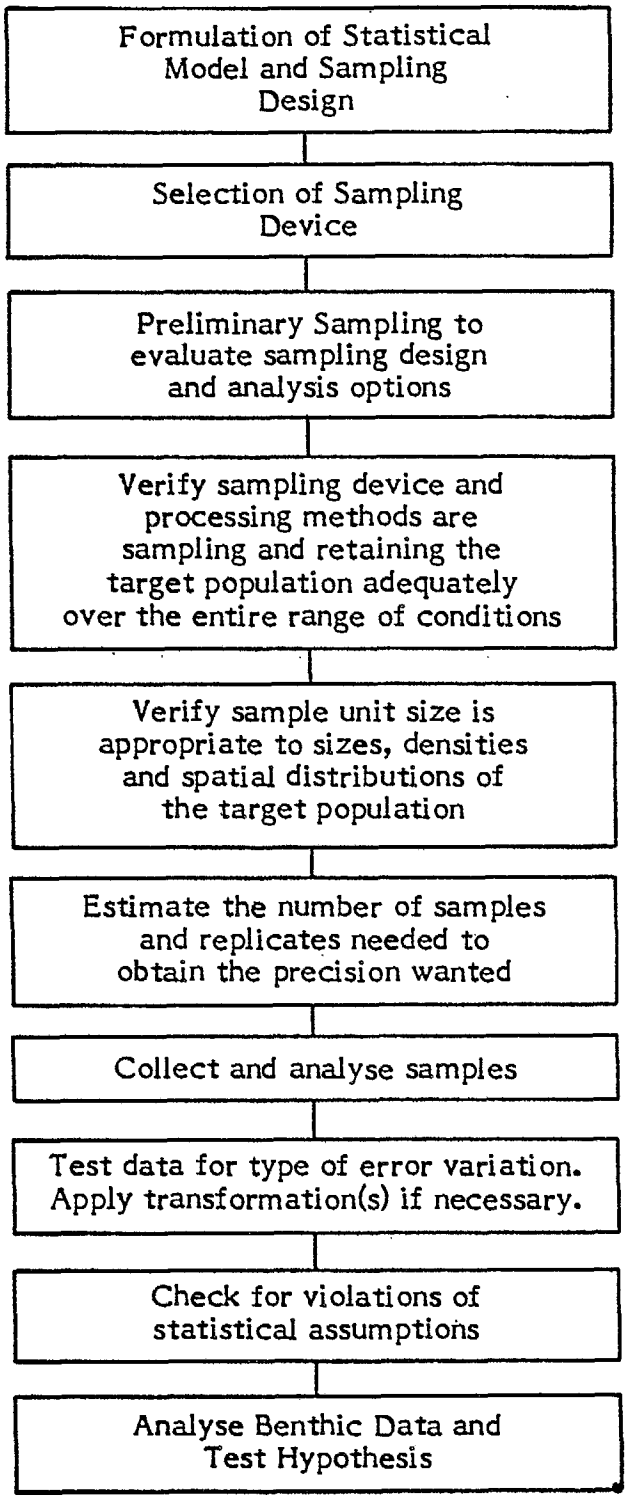


Figure 5.2. Sampling Design and Evaluation

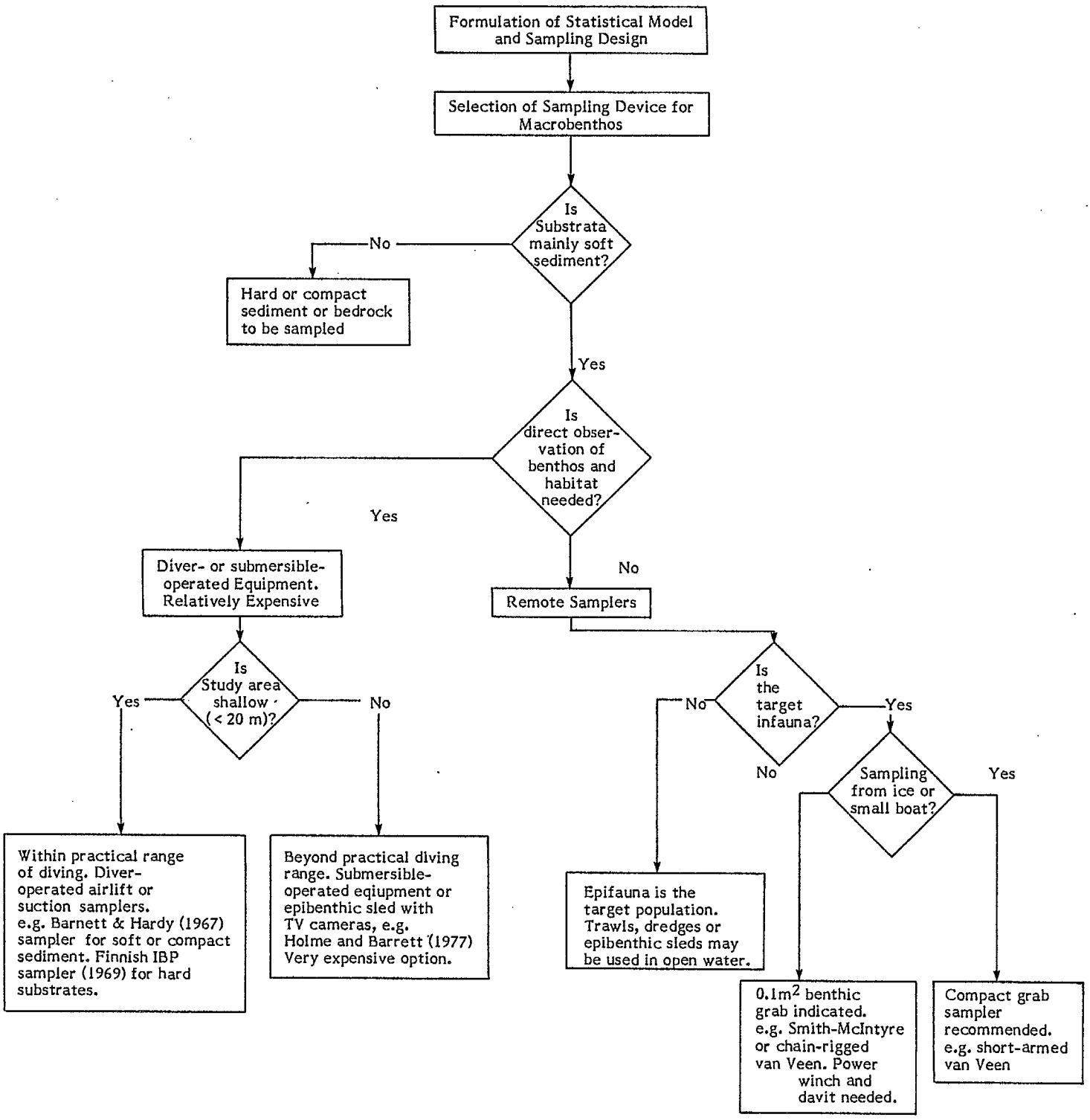


Figure 5.3 Selection of a Suitable Sampling Device for Macrobenthos Sampling.

Table 5.1

Summary of Methods of Macrobenthos Sampling

Sampling Device	Type of Information	Sampling Habitat	Organisms or Parameters	Typical Sampling Area	Unit of Measure	Limitations of Use	Approximate Cost	References
1. Grab	Quantitative	Soft or fine sediments	Infauna; biomass, density	0.1 or $0.2m^2$	$g\text{-}m^{-2}$ $no.\text{-}m^{-2}$	Soft sediment, limited area	van Veen $1500 Smith-McIntyre $5,000	Petersen 1977 Carey & Ruff 1977
2. Airlift	Quantitative with quadrat or cylinder	Hard or soft substrates	Epibenthos and infauna; biomass, density	0.05 to $1m^2$	$g\text{-}m^{-2}$ $no.\text{-}m^{-2}$	Shallow depths (<20m), and good visibility only; diver-operated	To fabricate $500 $1000	Barnett & Hardy 1967 Finnish IBP-PM Group 1969
3. Trawl	Qualitative (or quantitative with odometer)	Hard or soft even substrate	Epifauna, (biomass, density with odometer)	Unknown (or estimated from trawl odometer)	Comparative (or $g\text{-}m^{-2}$ $no.\text{-}m^{-2}$)	Open water, free of obstructions; smooth bottom	Beam trawl $2000-$3000	Carey & Ruff 1977 Carney & Carey 1980
4. Dredge	Qualitative or semi-quantitative	Hard or soft substrate	Epibenthos	Unknown	Comparative	Open water, clear of ice	Rectangular dredge $1000	Mohr et al 1957 Lee 1973
5. Epibenthic sled	Qualitative or quantitative with 6. and 7.	Hard or soft substrate	Epibenthos; density, biomass	Estimated from tow duration	$no.\text{-}m^{-2}$ $g\text{-}m^{-2}$	Open water, free of obstructions	Sled $1000-$2000	Holme & Barrett 1977
6. Still camera	Qualitative or quantitative with 3 or 5.	Hard or soft substrate	Epifauna; density	Various (e.g. 0.5 - $1m^2$)	$no.\text{-}m^{-2}$	Good visibility only; only with other devices (3,5)	Diver camera $1000-$3000 Remote camera $3000-$5000	Johnston et al 1969 Cross & Thomson 1981 Dyer et al 1982
7. Television	Qualitative or quantitative with 3 or 5.	Hard or soft bottom	Epifauna, density	Various	$no.\text{-}m^{-2}$	Good visibility only; only with other devices (3,5)	Black/White $1000-$10000 Colour $17000-$20000	Holme & Barrett 1977

randomly allocated sampling for each combination of location and time is presented by DeMarch (1976). Sokal and Rohlf (1973) have discussed and illustrated the principle of replication in relation to the concept of the F-statistic and its multivariate analogues. Pooling or combining of replicate samples eliminates the basis for estimating variation within and therefore should not be done except under special circumstances (see Brown and Fisher, 1972; Rohde, 1976).

If the study area has a large-scale pattern of environmental conditions, stratified sampling may be appropriate. If density variation is of principal concern, the area can be divided into relatively homogeneous subareas or strata within which the samples are randomly allocated in proportion to the size of the stratum. For example, if the study area has soft muddy sediments interspersed with gravel bars which account for one-fifth of the surface area of the local sea bottom, then the two subareas of widely different sediment types can be treated as strata with sample representation in the ratio 4 mud: 1 gravel in the sampling design. The variation in benthos abundance (or other criterion variables) between the strata can then be removed from the analysis, resulting in only the variation within strata entering the error variation term of the appropriate statistical test. Steel and Torrie (1960) and Cochran (1963, 1977) have described examples of stratified sampling with an estimation of the gain in efficiency of the statistical analysis from the use of a stratified as compared with a completely random design.

5.2.4 Preliminary Sampling and Background Data Acquisition

As indicated above, selection of an appropriate sampling design for a field study requires certain preliminary or background information on environmental conditions. In general, the more information available on an area before designing a sampling program, the better the sampling and its results. For proper planning of quantitative benthic studies, the following information is needed:

(1) Bathymetric data for the study area from a nautical chart or echo sounding surveys (summarized on a map of the area).

(2) Sediment data from all available sources (e.g. nautical charts, geological reports etc.). This information, in addition to data on the distributions of distinct water masses within the area, is one of the most important elements in planning quantitative benthic sampling (see Section 5.2.3).

(3) Oceanographic data on distribution of water masses and their movements, and tropic conditions in the pelagic environment of the study area. These data are necessary for planning and for data interpretation.

(4) Baseline information on chemical parameters if pollution studies are to be undertaken. This is necessary for selection of control or reference areas and delineation of the study area.

(5) Qualitative data on the types of benthic communities and their composition.

All of the existing information should be compiled and updated or extended by preliminary sampling fo the benthos and substrate. Preliminary sampling is especially useful for identifying potential sampling problems and for evaluating the sampling methods and statistical analysis options. On the basis of preliminary sampling, the number of samples needed to obtain a desired level of confidence can be estimated. This topic is covered in Section II - 3.1.3, Volume I and in general references such as Cochran (1977), Elliott (1977) or Green (1979).

Preliminary results may also be used to test for the type of error variation. Decisions in the use of transformations or nonparametric tests can be made on this basis. An examination of preliminary data for possible violation of assumptions of statistical methods can greatly aid in the choice of the appropriate statistical options. Basic references on the assumptions of classical statistical methods and the consequences of their violation are Eisenhart (1947) and Cochran (1947). The subject has been reviewed by Glass et al. (1972), with emphasis on the consequences of specified violations in simulated data. Their conclusions on the robustness of many statistical methods to violations of assumptions are reassuring. They conclude that correlated errors can have more serious consequences on the validity of statistical tests than all other violations. (Random sampling, at least at some level of sampling design, will prevent that violation.) If the errors are positively correlated, the test will tend to be more liberal than the stated level, whereas it will be more conservative if the errors are negatively correlated (Glass et al., 1972).

5.2.5 Statistical Analysis

The statistical model should be determined by the choice of the hypothesis model. The analysis used depends, therefore, on the information required to test the

hypotheses and on the sampling design. For this reason, it is critical that the purpose of the study and the expected methods of analysis are established in the planning stage rather than after all of the samples are collected.

In addition to being compatible with the hypothesis model, the chosen type of statistical analysis should be efficient, in other words, be as conservative, powerful and robust as possible. Being conservative implies having a low probability α of making the Type I error of incorrectly rejecting H_o. Being powerful means having a low probability β of making the Type II error of accepting H_o when it is false. Being robust signifies that the stated error levels will not be seriously affected by the kinds of data commonly collected in environmental studies, which rarely satisfy all of the assumptions of statistical models (cf. Section 5.2.3). Robustness of statistical inference will be increased by the use of several analysis methods based on different assumptions. If the methods, despite their different assumptions, lead to similar results, then the decisions will be robust or relatively free of the assumptions of the methods. This approach can be particularly useful where performing exploratory analyses on multivariate data sets where hypotheses are poorly defined (e.g. Section 5.2.1) (Green, 1979). For example, a null hypothesis that there are no natural groups or clusters in the samples might suggest the use of cluster analysis or ordination procedures. If several clustering or ordination techniques, based on independent algorithms, all produce the same grouping of samples, then the decision that there are real groups of some kind is a robust one.

5.3 Classification of Applied Biological Studies

Applied environmental studies generally can be divided into three major classes: baseline, monitoring and impact studies. Impact studies have several variations as well (Section 5.3.1). These terms have been used loosely in the literature, but the definitions given below, after Green (1979), are used in this guide.

(a) Baseline Studies

A baseline study has a descriptive goal (Section 3.1) and is done to define or describe the present ("baseline") state of the biological community, the environment, or both. Generally some environmental change is projected, but the nature of the

change and its timing may be unknown. If spatial and temporal (e.g. seasonal) variation of biotic and environmental variables occur in the system, they should be represented in the baseline model (e.g. Section 5.3.1.2).

(b) Impact Studies

An impact study has the objective of determining whether a specified impact affects a biological community, and if so, to describe the nature of the change. It may be possible to collect both before- and after-impact data (permitting an optimal impact study design) or only after-impact data. There may or may not be a control or reference area which does not receive the impact. **The nature of the impact and the certainty of its occurrence is always known.**

(c) Monitoring Studies

A monitoring study has the objective of sampling to detect a change from the present state. If detection of a change in a biological community is involved, then it is a biological monitoring study. Baseline data must have been collected to provide a standard against which a change might be detected. To permit the highest sensitivity of monitoring studies in detecting effects, the change should be clearly defined by using the results from earlier impact studies on the same or similar system. This definition provides a specific alternate hypothesis against which to test the null hypothesis of no detectable change.

5.3.1 Impact Study Designs

An impact study is best designed when it contrasts impact effects to previously collected baseline data. It's highest or optimal level of use is achieved when the results provide the basis for subsequent monitoring to detect future impacts of the same type.

An optimal impact study design has four requirements:

1. The impact must not have yet occurred; before-impact baseline data thus establishes a temporal control against which the after-impact data can be judged.

2. The type of impact and its timing and location must be known so that a sampling design suitable for tests of hypotheses can be devised. This property distinguishes it from a monitoring study to detect change.

3. Measurements of all relevant biological and environmental variables must be obtainable in association with the individual samples to permit hypothesis testing.

4. A non-impacted area must be available to serve as a spatial control or reference area.

Reference standards in both time and space are necessary for an optimal impact study design because evidence for impact effects on the biological community must be based upon changes detected in the impact area that were not observed in the reference area. Impact studies which lack one or more of the above requirements are "suboptimal."

A flow chart (Figure 5.4) conveniently illustrates the distinctions between five categories of applied biological studies. The distinctions are based on three decisions regarding design prerequisites. The questions are (a) whether the impact has already occurred; (b) when and where it occurred; and (c) whether a control area is available. From the responses it is possible to decide which category of study design is appropriate in a given situation. In this scheme, baseline and monitoring studies are combined in one category since the specific time and place of range from the present state are unknown. Notice that categories 2 and 4 are suboptimal impact studies which lack a spatial or a temporal control, respectively. Category 5 is the worst case, lacking controls and information on origins or the impact. The last category is rather uncommon and requires specialized strategies. Principal emphasis is on the first four categories. Within each of the five study categories, the general planning steps discussed in Section 5.2 are applicable. More specific recommendations for each study category are given in following sections.

5.3.1.1 Optimal Impact Study Design and Analysis

If an optimal impact study design is indicated by the decisions in Figure 5.4, then the options for sampling design and statistical analysis will be similar to those outlined in Figure 5.5. Due to its requirements for sampling multiple areas and times, an optimal impact study design is necessarily an area-by-times factorial design in which the evidence for impact effects is a significant areas-by-times interaction.

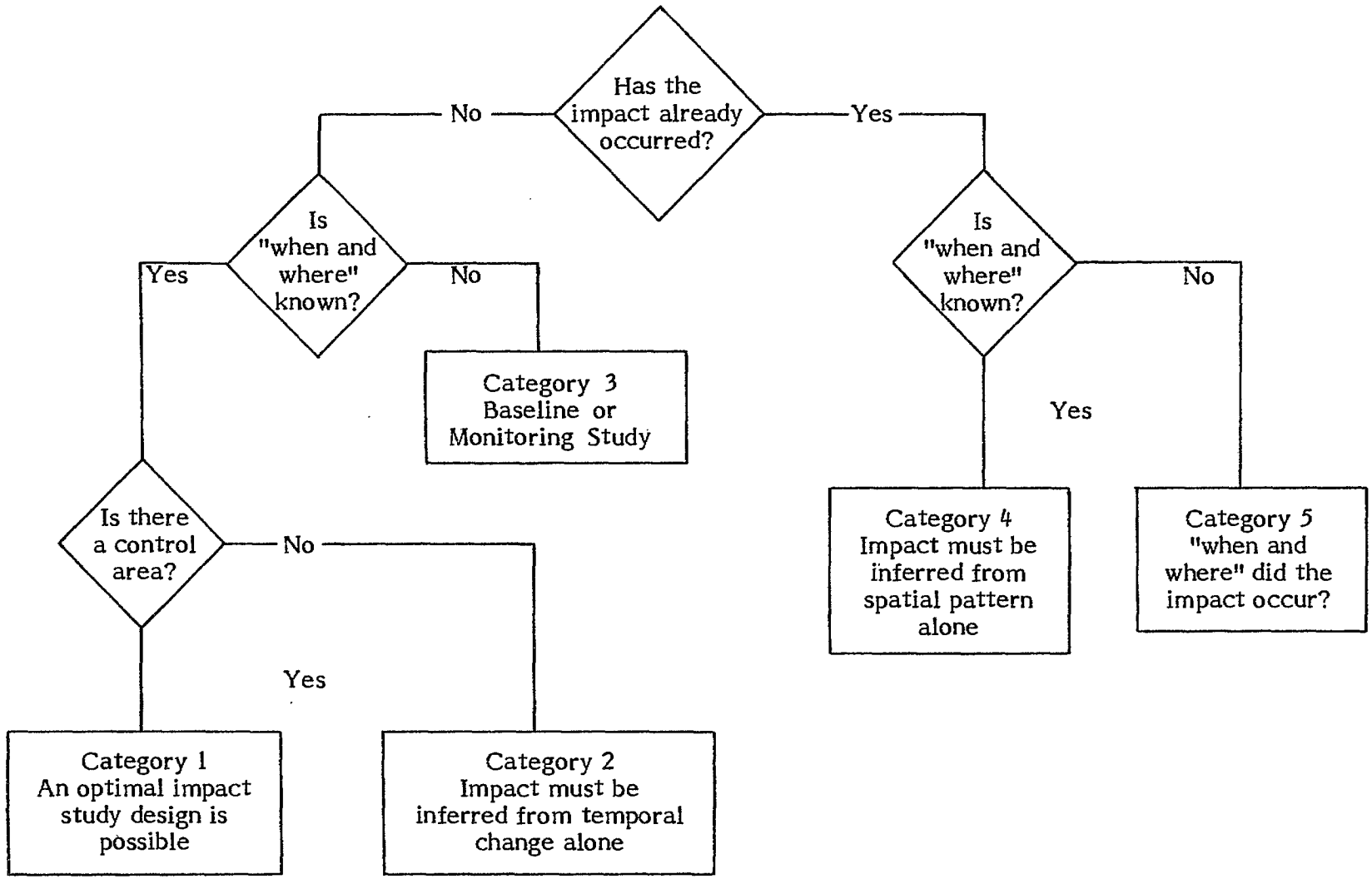


Figure 5.4 Flowchart for decisions on the major categories of applied biological studies (after Green , 1979).

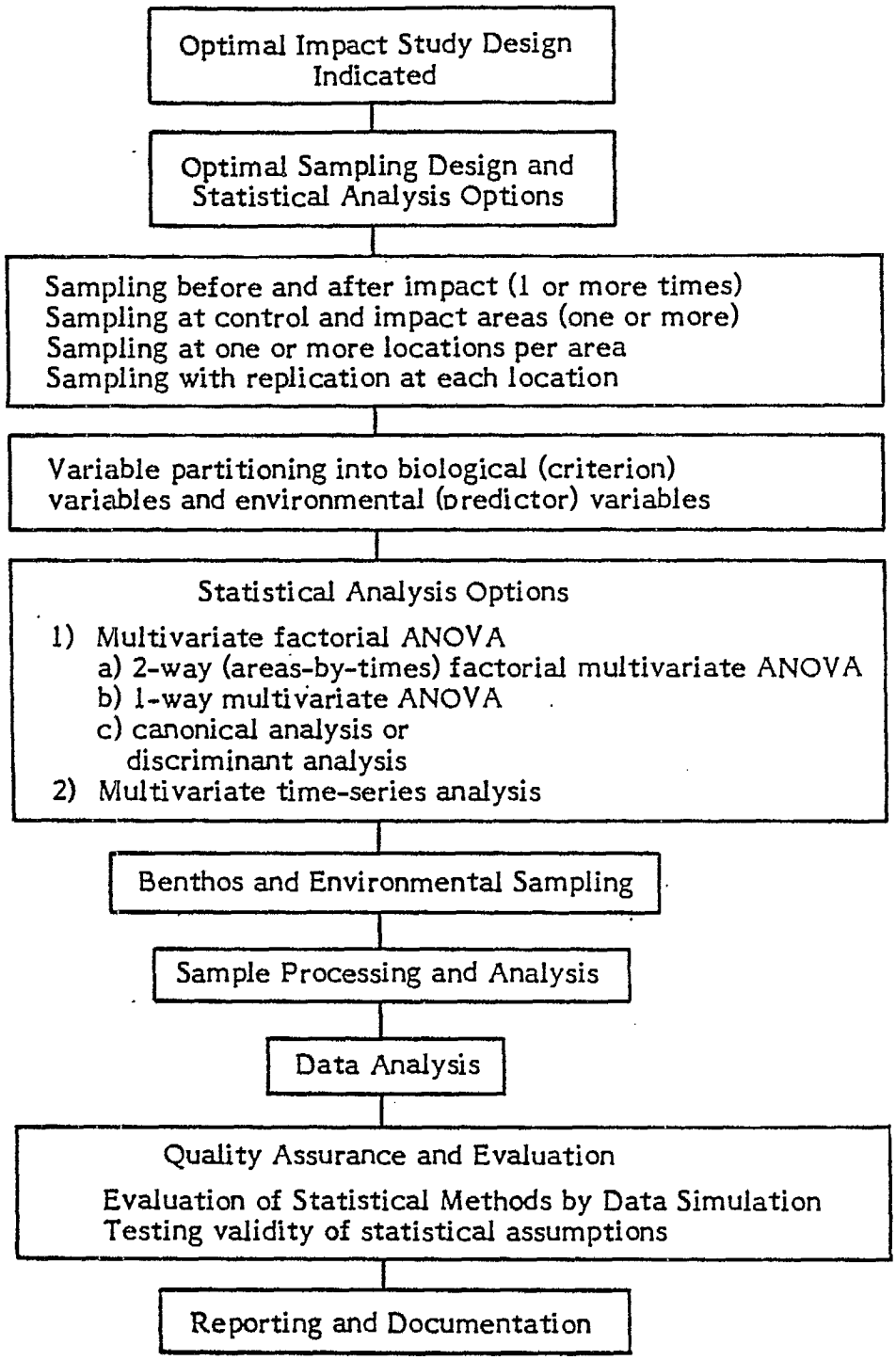


Figure 5.5. Category 1: Optimal Impact Study Design

As suggested by the ANOVA terms used, ANOVA is a highly appropriate method for statistical analysis of an optimal impact study. This method and the other options in Figure 5.5 will be discussed later in this section.

The final choice of a particular sampling design and statistical procedure should be based on the following criteria:

1. There must be a testable null hypothesis that any change in the biological community of the impact area, over a time interval which includes the impact, does not differ from a control or reference area.

2. One should be able to relate to the impact any significant change unique to the impact area and to separate effects caused by natural environmental variation not connected with the impact.

3. The method of data analysis should be conducive to an effective visual representation of both the effects due to impact in relation to other sources of variation and the relationship between impact-related change in the biological (criterion) variables and input-related change in the predictor variables.

4. The results of the analysis should be suitable for later use in biological monitoring to detect future impacts of the same type.

5. The test of the null hypothesis that no change due to impact occurred should be as conservative, powerful and robust as possible (see Section 5.2.4).

Multivariate factorial ANOVA methods are often well suited for application to the optimal impact study situation (Figure 5.5). In the two-way (areas-by-times) factorial multivariate ANOVA design, the test of the null hypothesis of no impact effects in the biological community corresponds to the test of the null hypothesis of no area-by-times interaction for the biological variables. The equivalent null hypothesis may be tested for impact effects on environmental conditions by using the environmental variables. Samples may be allocated within the four areas-by-times combinations in either of the following ways: (a) randomly or (b) randomly within each of a number of randomly positioned locations nested within the areas-by-times combinations. An example of (b) for sampling of benthos might involve random nested sampling by grab (e.g. randomly allocated replicate sampling) at randomly positioned locations within the impacted and non-impacted areas at times before and after the impact occurs. Sampling design (b) permits accounting for among-location-within-area variability caused by a heterogeneous environment. A further example of

nesting might involve random subsampling of the grab samples with small corers (e.g. Hamilton et al., 1970). Examples of applications of higher order multivariate ANOVA designs are presented by Mager (1974) and Harris (1975).

If the sampling locations are randomly allocated in each area for the initial before-impact sampling, and then the same locations are sampled each subsequent time, then the data will be in a form suitable for analysis by one-way multivariate ANOVA. (Note that in the factorial design, sample locations are randomly allocated in each of the four areas-by-times combinations.) An example of a one-way multivariate ANOVA design to detect impact effects on a benthic species assemblage is to sample randomly allocated locations in the impact and control areas to obtain two groups of data on changes in species abundance variables (e.g. percentage change; Green, 1979). A one-way multivariate ANOVA would test the null hypothesis that the mean values of the species change variables for members of the species assemblage do not differ between the two areas. This design is similar to that used in a paired t-test. Green (1977, 1979) has presented an example for benthic communities based on this design, but lacking a control area.

Advantages of the one-way design over the factorial design include:

(a) simpler sampling and analysis design.

(b) computer programs for statistical analysis are more widely available for one-way multivariate ANOVA.

(c) alternatives are available for one-way ANOVA for remedying violations of statistical assumptions (e.g. homogeneity of within-area variance-covariance matrices), such as nonparametric procedures (Kendall, 1966; Mantel, 1970). Nonparametric analogues have not been developed for factorial designs.

(d) the possibility of regressing the species change variables or environmental change variables exist for one-way designs but not for factorial designs.

On the other hand, the disadvantages of the one-way analytical design in relation to the factorial design include:

(a) in the one-way design, the random sample is equivalent to the random location, which is fixed for pre- and post-impact sampling times. The relative influence of an odd or atypical location may be increased since it will be sampled twice during the study.

(b) conversion of species abundances to differences will tend to increase the sampling error because the variance of a difference equals the sum of the variances of the raw values.

(c) the one-way analytical design does not correspond exactly to the factorial sampling design. The factorial ANOVA design, however, has a direct and logical correspondence with the optimal impact study design.

Multivariate ANOVA and related subjects such as canonical analysis and discriminant analysis have been reviewed by Cooley and Lohnes (1962, 1971), Marriott (1974) and Harris (1975). Green (1979) has presented a worked example of canonical analysis, which is closely allied to multivariate ANOVA, in an optimal impact study on simulated benthic data. An advantage of canonical analysis is that critical values of the canonical variates can be used as possible monitoring criteria for detecting future impacts in the system. This technique will be discussed in more detail in Section 5.3.1.3.

Another approach to optimal impact study design is that of multivariate time-series analysis, a term which covers a broad group of techniques. Basic references on these methods are by Anderson (1963, 1971), Munn (1970) and Batschelet (1976). This methodology requires a series of observations over time, adequate to demonstrate the nature of the pre-impact temporal pattern of the studied system. After a specified time when impact occurs, the null hypothesis of no change in this temporal pattern may be tested. Optimal sampling design requires that a control or reference area be sampled and analyzed in a parallel fashion. For environments with marked seasonal variation and with impacts occurring over extended periods and areas, the approach of time-series analysis has considerable merit. The major drawback is that such studies need specialized temporal sampling designs with a large number of samples, and consequently a large operating budget, especially if the sampling is done in remote areas. Ideally, the ANOVA sampling design and analysis based on two sampling times circumvents rather than follows any natural temporal variation by isolating the impact in time through sampling done just before and soon after the event. In cases where the impact occurs over an extended period of time, the results of the multivariate ANOVA can be included in a monitoring study (Section 5.3.1.3).

5.3.1.2 Suboptimal Impact Study Design:
Impact Inferred from Temporal Change Alone

In this situation a control or reference area defined at the design or planning stage is missing. The options for suboptimal sampling design and analysis are outlined in Figure 5.6. If sampling is done at different times at a number of different locations, then temporal change in spatial pattern of the variables can be the criterion of impact. However, if there is only one location (impact site), then time-series analysis modelling must be used (e.g. Hannan, 1960, 1970; Hamon and Hannan, 1963). The most common statistical analysis model used to relate biological variables to environmental factors when there is no control area is multivariate ANOVA with canonical analysis for the multivariate case, and multiple regression and correlation for the univariate case (Figure 5.6). Green (1977, 1979) has described a detailed simulation example of a multivariate sampling design and statistical analysis for the situation where benthic samples at an impact area were spatially allocated at sites on a grid for single times before and after impact. The statistical model chosen assumed a linear additive relationship between changes in species abundance and changes in environmental variables, over a short time interval. In addition, by applying a logarithmic transformation to the data, one implies in this model that percentage change rates are linearly and additively related. The result is that spatial, within-time variation in criterion and predictor variables is removed from the analysis. Since the sampling stations are paired across time, the design is analogous to the use of a paired t-test for comparing the means of two populations where there is high within-group variation and a logarithmical pairing of individuals between the groups (Green, 1979). If sampling at all stations is repeated more than two times, the above statistical design can be easily modified. For instance, instead of using the difference in each variable between the two times, one could calculate the average change in each variable per unit time at each station. The additional sampling would allow even more sensitive tests of hypotheses regarding the effect of the impact on species abundances.

Another approach to the analysis of change in spatial pattern of species composition over a series of sampling times is to apply ordination or cluster analysis to the successive groups of data (eg. Williams et al. 1969). These methods are discussed in Sections III - 6.2.4.2 and III - 6.2.4.3 of Volume I. This analytical approach can be effectively used where an impact occurred at a certain time and location and its effects on the biological community persisted, possibly with spatial changes too.

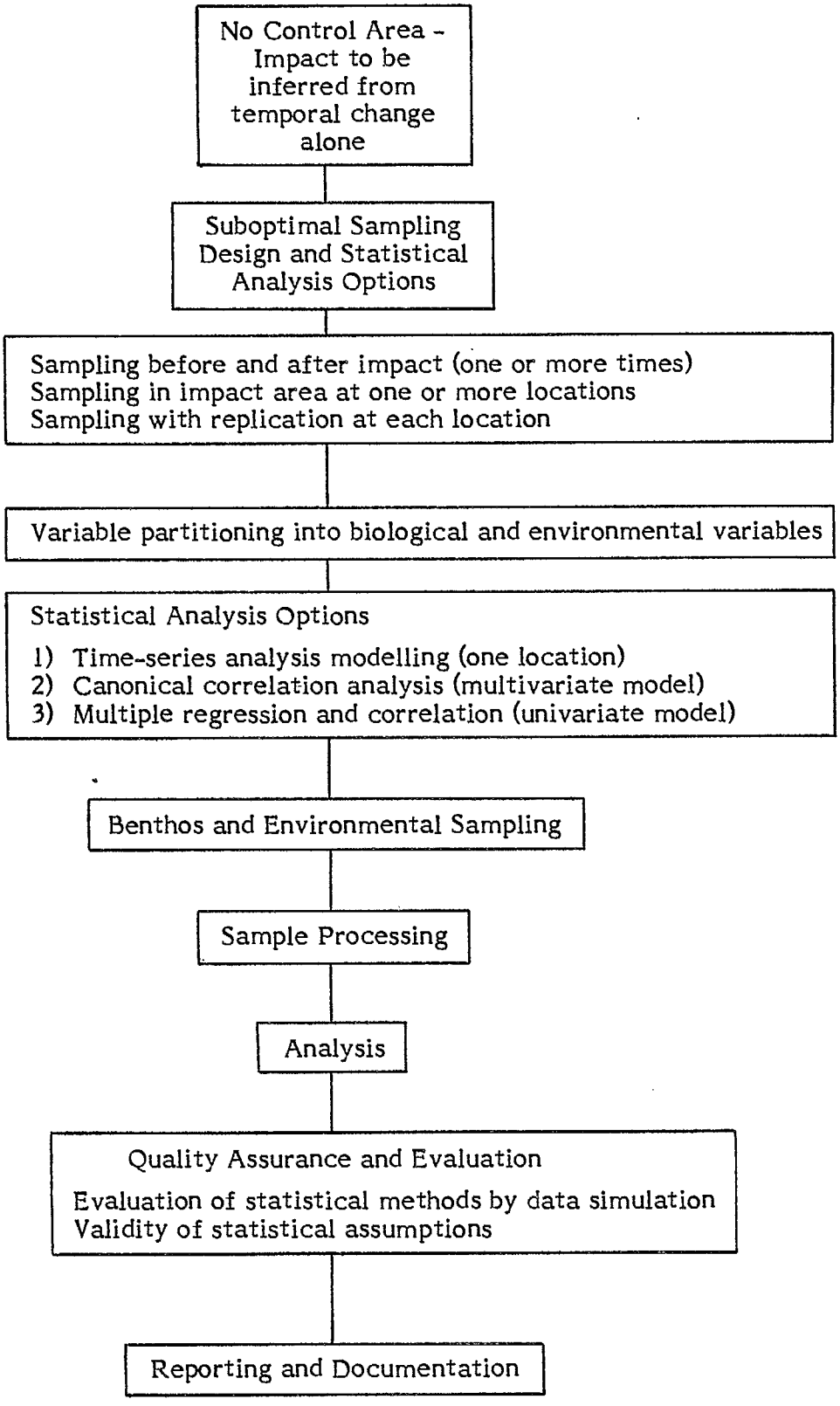


Figure 5.6 Category 2: Impact Inferred from Temporal Change Alone.

Time series analysis methods (Anderson 1963, 1971; Section 5.3.1.1) can be applied in suboptimal impact studies which lack a control area if sampling is repeated several times. Time series analysis can be done with binary as well as quantitative data. Cox and Lewis (1966) and Davis (1973) have described time series analysis with binary data. A data set (for example, species counts over a series of observations), can be tested for variation in species success (presence) among times by a procedure, such as Cochran's (1950) Q-test. This test has been described by Siegel (1956) and illustrated with a worked example by Green (1979).

For a suboptimal impact study involving collection of a large set of time series species data, an option would be to test the null hypothesis that "nothing is going on in this time series of binary species data" by Cochran's Q-test, followed by ordination or cluster analysis to describe the temporal changes in the data if the null hypothesis is rejected. For instance, ordination techniques applied to data sets where the number of species greatly outnumbers the sampling times would permit the representation of temporal species-groups in a reduced space (see Section III - 6.2.4.3 of Volume I). The temporal species assemblages could be linked by arrows to show the direction of movement through the ordination space. Alternatively, cluster analysis could be applied through the method of association analysis (Williams and Lambert 1959, 1960; Green and Hobson 1970) which could be used to identify the best indicator species for further monitoring.

As mentioned in Section 5.3 (a), temporal variation (eg. seasonal or other types of cycles) should be represented in baseline and impact study models. Livingston (1976, 1977) has treated the topics of seasonal, tidal and diurnal cycles in estuarine systems as related to pollution studies. Durbin (1963) and Bulmer (1974) have described methods for estimation of seasonal and other cyclical components in time series of species data.

5.3.1.3 Baseline or Monitoring Study Designs

For studies in this group, an impact has not yet occurred and when, where or even if an impact will occur is not established (Figure 5.4). Most frequently, sampling will be done at one or more locations within one area to detect impact changes if they occur (Figure 5.7). If a control or reference area and a potentially impacted area can be established, then sampling will be performed at more than one location per area.

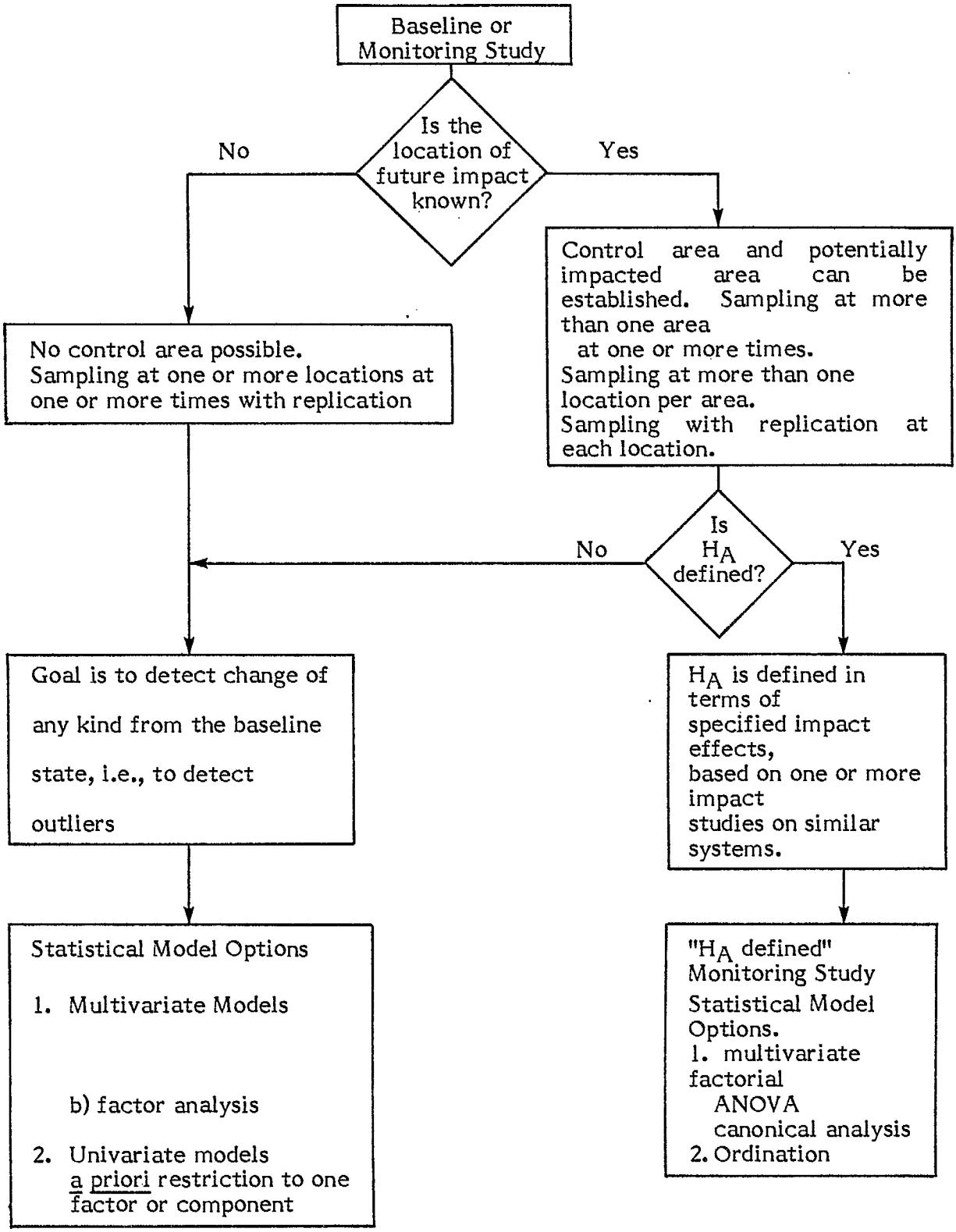


Figure 5.7. Category 3: Baseline or Monitoring Study. H_A refers to the "alternate hypothesis" to the null hypothesis, H_0 (Section 5.2.1).

Any monitoring study requires baseline data because impact changes can only be identified by departure from the unimpacted or reference state. The time interval and amount of detail necessary for description of the baseline state will depend on the degree of cyclical behaviour and other natural variation in that state (see Section 5.3.1.2 for a treatment of time series data).

A major decision in the design of baseline or monitoring studies is determining whether or not a specific alternate hypothesis (H_A) can be defined in terms of particular impact effects which are in contrast to the null hypothesis (H_o) that baseline conditions of the biota are unchanged (Figure 5.7).

If the biological response to the impact is uncertain, because either the nature of the impact is unknown, or it has never occurred previously, then the only appropriate H_o is that there is no biological change to the baseline state. If this H_o is rejected, then the aim is to detect change of any kind in the biological community from the baseline state. The various options in choosing statistical models for this situation include multivariate techniques such as ordination, factor analysis and clustering (see Section 6.2.4 of Volume I) and univariate models when there is a restriction to one pair of indicator and predictor variables in the design. The basic strategy is to detect outliers in the data which might indicate that a change from the baseline state has occurred. An example is to determine whether a new observation on species abundance belongs to the multivariate normal baseline distribution of the before impact data. An effective approach is to reduce the multidimensional data to a two-dimensional space by principal components ordination and to construct confidence ellipses (Sokal and Rohlf, 1969). The position of the new observation on species abundances relative to the confidences ellipses will indicate whether the observation is an outlier or not. Green (1979) has given a worked example of this approach.

A much more satisfactory situation for design of a monitoring study is to have an H_A defined in terms of specified impact effects. This kind of H_A requires not only information on baseline conditions, but also information about the biological response to the particular impact, based on one or more impact studies on the same or similar systems. For example, such an "H_A defined" monitoring study to detect future impacts of the same kind might be based on a previously conducted optimal impact study. Green (1979) has provided an example of a biological monitoring study based on an optimal impact study of benthic communities. He used the canonical variate associated with species interaction (CVSI) derived from canonical analysis for the biological criterion to detect future impacts of a similar type. From samples

collected at several times subsequent to the impact a CVSI score was calculated from the species abundances of three species. If the estimated CVSI score exceeded a specified critical value then a possible impact would be indicated. The critical value of the CVSI score can be chosen from a cumulative percentage plot of unimpacted and impacted CVSI sample values on arithmetic probability paper (Green 1979).

5.3.1.4 Suboptimal Impact Study Design:
Impact Inferred from Spatial Pattern Alone

When an impact study is initiated after the impact has occurred, then its effects must be shown and described from the spatial distributions of the biological and environmental variables (Figure 5.8). Sampling design may involve sampling at more than one location within one or more areas on one or more occasions. However, because sampling areas differing in degree of impact cannot be selected beforehand, and spatial rather than temporal pattern must be the key source of information, the most frequently used sampling design in this situation is the following:

(a) sampling on one occasion at more than one location within the impacted area, with replication at each location.

(b) samples for biological variables have corresponding observations on environmental variables.

For the statistical analysis of the spatial pattern of the variables, a two-staged procedure is recommended:

(1) reduce the biological variables (e.g. species abundances) to fewer variables in a smaller dimensional space by methods such as ordination or clustering (see Section III - 6.2.4 of Volume 1), and

(2) relate the derived biological variables to environmental variables to account for the variation in the biological variables.

Ordination procedures will reduce the biological data to a smaller number of continuous biological variables which can then be related to the environmental data

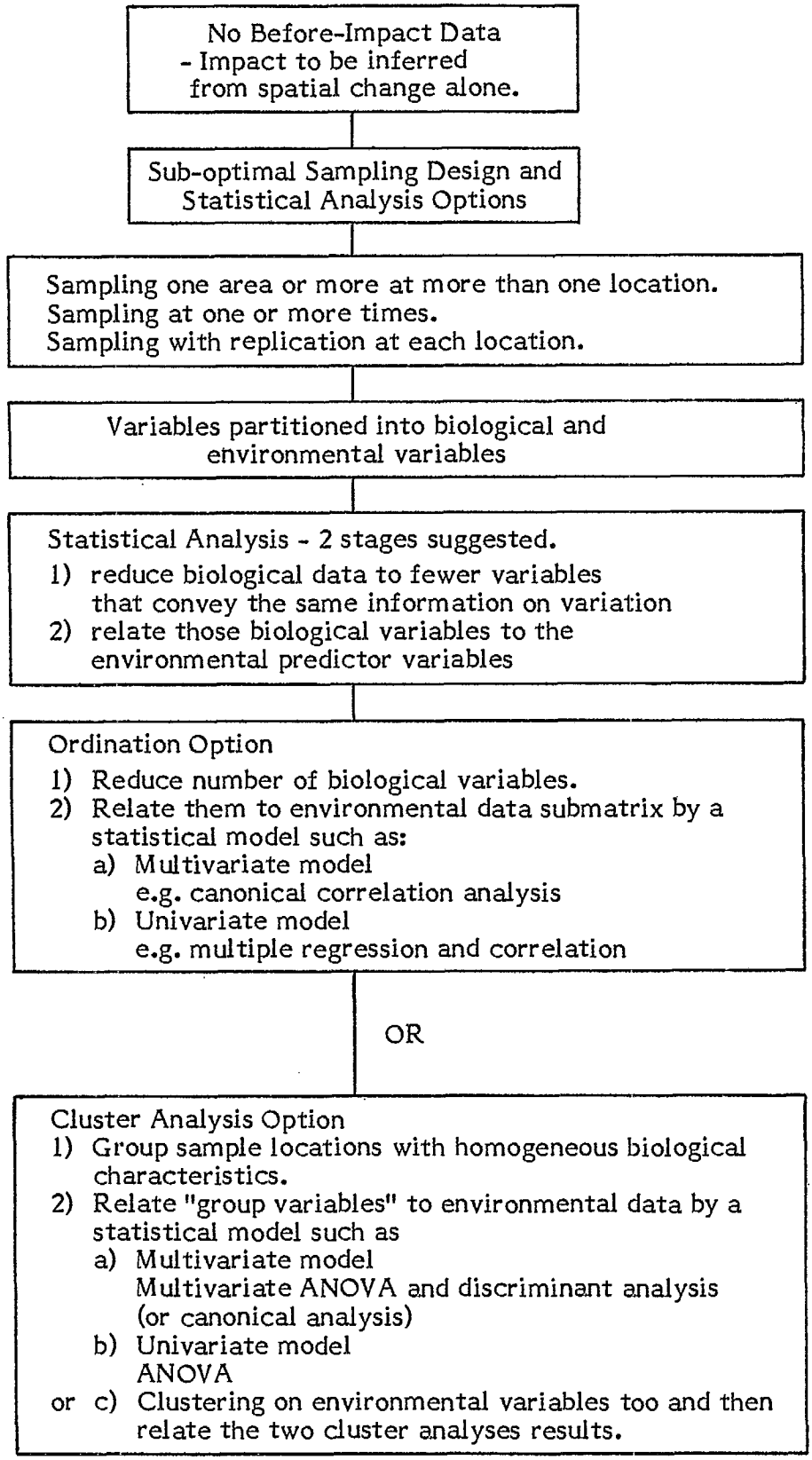


Figure 5.8 Category 4: Impact Must be Inferred from Spatial Pattern Alone.

through a statistical model. For multivariate cases, cannonical or multiple discriminant analysis (e.g., Cooley and Lohnes, 1962, 1971; Pimentel, 1978; Green, 1974, 1979) might be appropriate. In a univariate analysis, multiple regression and correlation might be applicable. Note, however, that the assumption of a linear relationship between population success and environmental factors over the natural range of the variables and during extended time intervals is often unrealistic due to the unimodal rather than linear distributions of species on environmental gradients. When using statistical models which assume linear relationships between variables (e.g., canonical correlation, factor analysis, linear or multiple regression) it is important that the linear model be applied among the environmental variables rather than among the biological variables, or between the biological variables and the environmental variables. For example, in multiple discriminant analysis (or canonical analysis), the discriminant functions used to evaluate and interpret the separation of groups of entities assume linear relationships between selected environmental variables (e.g., temperature, salinity or depth) which are logarithmically transformed if necessary. The distribution of the biotic variables (e.g., species assemblages from clustering or ordination) on the environmental variables is assumed to be nearly multivariate normal. The validity of the latter assumption, however, is not critical due to the unimodal character of biotic distributions (Green, 1979).

Another approach for relating biotic variables to environmental variables is to perform cluster analyses on the environmental factors as well as the species data (e.g., Sandilands, 1977). The results of the two cluster analyses could then be compared by methods described by Rohlf (1974), such as two-way contingency table analysis.

Whatever the methods selected for examining impact effects through analysis of spatial pattern, it must be recognized that in the absence of a before-impact temporal reference, that the inferences derived always depend on the untestable assumption that the observed spatial pattern is influenced by the impact and did not exist before the impact. In addition, results from spatial pattern analysis, especially those involving transects across environmental gradients, can be deceptive due to confounding of variables (e.g., strong intercorrelations between salinity, depth and substrate properties in transects between an estuary and open ocean; see Bird, 1970). Variation in distributions of species on environmental gradients may not be accounted for by environmental factors alone, even if all of the important ones are considered (Pielou, 1974, 1975). For example, the effect of intrinsic biological variation such as interspecific competition may produce complex species patterns along environmental gradients which could be mistakenly attributed to environmental variation in a descriptive study (Fenchel, 1975).

5.3.1.5 Category 5: When and Where Did the Impact Occur?

An applied environmental study which is initiated without knowing when or where the impact began represents the worst possible case. The aim here is to find out "when and where" and the possible degree of natural versus human causes of the changes. The approach to the problem must be to determine whether the effect existed before possible human causes were present or whether it now is evident in areas unaffected by the hypothesized human causes. The problems of mercury pollution (Katz, 1972) and chlorinated hydrocarbon residues in the food chain (Hickey and Anderson, 1968) are prime examples of impact studies of this type.

Although specific methods for study design and analysis cannot be recommended for this situation, it is noteworthy that technqiues for analysing growth rates over annual and shorter cycles have been developed for fossil shells (Panella et al., 1968). The use of such methods to investigate biological effects of past environmental variation might prove to be fruitful.

5.4 Sampling Methods for Macrobenthos

Appropriate methods for collection of macrobenthos will depend on several factors, such as the target population (infauna or epifauna), substrate type (soft mud to bedrock) and sampling platform (from the ice or a research vessel). A general review of methods and recommendations for remote and direct collection of macrobenthos are presented in Sections II-3, 4 and 5 of Volume 1. Selection of a suitable sampling device is treated as a component of sampling design (Section 5.2.3). This section will consider appropriate practices for collection and subsequent treatment of quantitative macrobenthos samples.

5.4.1 Recommended Practices for Remote Collection of Macroinfauna

The following practices are advised for quantitative remote sampling of macroinfauna from soft marine sediments:

1. Select a suitable grab sampler which collects a surface area of at least 0.1 m^2 (e.g., van Veen, Smith-McIntyre: see Section II-3.31 of Volume 1 for selection criteria).

2. Determine the number of samples necessary to adequately sample the number of species present, the distribution and abundance of the infauna (see Section II-3.1.3, of Volume 1).

3. In open water, the sampling vessel should be anchored on station if possible to prevent drifting, and to reduce the wire angle so that the grab strikes the sediment surface squarely and lifts vertically.

4. Use a light wire (4-6 mm diameter) of good quality and condition so that the chance of kinking or fouling is minimized. A strong swivel (e.g., stainless steel or bronze ball-bearing type) should be connected between the grab and the wire end to prevent twisting and kinking of the wire.

5. Sampling depths should be measured by echo sounder and/or meter-block. See Rees (1971) for deck arrangements. Setting down of the grab during lowering should be done as gently as possible in order to reduce the shock wave preceding the grab. Gentle immediate closing of the grab after impact should prevent the loss of sediment due to jerky lifting of the grab before complete closure of the jaws.

6. The volume of sediment collected with each cast should be measured in a graduated container and recorded in the sampling log. If less than 5 L of sediment is obtained, as may happen on firm sand, then the results should indicate a low sample volume was collected. For a sample to be acceptable the grab should be fully closed on retrieval and should contain a full bite of sediment. Samples with evidence of leakage of contents should not be used for quantitative results.

7. Adequate training and experience in the operation of the grab should be provided for sampling personnel before actual field sampling begins. There is a "learning curve" for grab operation (see Ursin, 1954).

8. Grab sampling through the ice with a grab of sufficient size imposes many restrictions. These translate into increased sampling time (and cost) compared to open water sampling from a research vessel. Grab sampling from the ice can be done with a compact grab (e.g., short-armed van Veen,

see Petersen, 1977) by using a hand winch mounted on a portable stand. It may be possible at some locations to take samples beside leads in the sea ice (e.g. Curtis, 1972). Otherwise it will be necesssary to cut a hole in the ice which is large enough to deploy the cocked grab. Lowering the grab sideways rather than upright through the hole may be possible but is more difficult and will often result in premature tripping of the grab. Even with a "short armed" Van Veen, a hole of 80 cm will be required. A hot water drill or melter is therefore most suitable (Section 4.3.2). When replicate samples are taken, the line should be kept in different corners of the hole for each sample to prevent overlapping of grab bites. If overlapping is suspected, separate holes may be required for each grab. When sampling from a lead, previously disturbed bottom can be avoided by moving the winch and stand along the lead between samples. The choice of locations for sampling from the ice, as suggested above, will be highly dependent on ice conditions. Adverse conditions such as the presence of pressure ridges, rubble fields, open leads, or rotten ice will often dictate where sampling may or may not be done. It is, therefore, recommended that contingency plans be devised in order to satisfy the essential criteria for choice of sampling location, whether they be water depth, distance from a reference point or random positioning within a sampling grid. Benthos sampling from the ice is slow, hard work. Allowance should be made in the ice sampling schedule for several more hours per station than for open water sampling to account for transportation of gear to the station, preparing suitable size ice hole(s), and manual sampling operations under severe conditions.

5.4.2 Recommended Practices for Diver Collection of Macrobenthos

Diver-operated sampling techniques are especially suited to studies in areas with hard or gravelly substrates and uneven surfaces. On level, soft sediments diving can also be used but it has fewer advantages over remote sampling. The ability to make direct observations on the benthos and their habitat is a principal advantage, whereas relatively high cost and depth or visibility limitations are major drawbacks. Diving applications and suitable gear for cold water use are described in Section 4 of Volume I.

For quantitative diver sampling of macrobenthos the following practices are suggested:

1) For quadrat sampling, select an airlift sampler suitable for operation on the type of substrate and in the depth range to be encountered in the study area (e.g., Finnish IBP-PM (1969) suction sampler for level hard substrates, in 2-15 m of water; see also Section 5.2.3 of this guide and Section 4.1.4.1 of Volume I for details and references on the equipment available and their modes of operation). For shallow (< 20 m) study areas in which both hard substrates and firm sandy sediments occur, it would be desirable to equip an airlift tube with the funnel of the Finnish sampler for the hard substrates and the coring tube of the Barnett and Hardy (1967) sampler for sandy or soft sediments.

2) Collect the benthos samples as indicated in the cited descriptions, keeping replicate samples separate for processing and analysis. To achieve this, easily detachable sample collectors or more than one sampler may be used to minimize loss of working time between collection of replicate samples.

3) If line transects are used to sample a study area, then the techniques of in situ counting, quadrat sampling and quantitative diver operated still photography may be used in conjunction with the transect to representatively sample the range of macrobenthos present (see Section 4.1.4.1 of Volume I).

5.4.3 Dredging and Trawling

Dredges and trawls are generally used for collecting epibenthos and demersal fish, cephalopods and crustaceans associated with or feeding on the sea bottom. General references on these samplers are Gunter (1957), Holme (1964) and Holme and McIntyre (1971). Section 3.2.1 of Volume I presents a review of these devices and their modes of operation. **Dredging and trawling in arctic waters are restricted to open water in areas relatively free of floating ice.**

The following practices are advised for sampling by biological dredge:

1) A weak link which breaks first under heavy strain due to a hangup on the bottom should be used to prevent loss of gear and to improve safety of operation (see Section 3.2.1 of Volume I).

2) The dredge should be towed slowly and steadily (0.5 to 1 m/sec) on a single 11 mm diameter wire rope of 6 x 19 construction in good quality and condition. A strong swivel should be placed between the dredge and the wire terminal to prevent twisting and kinking of the wire.

3) The surface area covered by the dredge is usually estimated from the length of time at the bottom depth multiplied by the speed of the tow and by the width of the dredge. Some recent dredges have metering devices to record the distance moved along the bottom (e.g., Martec/Shell).

For sampling macroepifauna by trawl, the following procedures are suggested.

1) The bottom topography of the study area should be examined for possible obstructions or hazards by consulting bathymetric charts or by running echo sounding transects along the routes of proposed sampling tows.

2) **A beam trawl with a 3-m mouth aperture is recommended over the otter trawl** for this purpose since the net gape is fixed by the beam whereas the spread of the otter boards and net wings is quite variable for the otter trawl. The mesh size of the net should be appropriate for retention of the target organisms.

3) The beam trawl should be towed at 1 m/sec from a single wire rope (11 mm diameter, 6 x 19 construction) of good quality and condition. A sturdy swivel should be inserted between the trawl bridle and the terminal of the timing warp to prevent twisting and kinking of the wire.

4) A bottom time of 10 minutes per tow and two tows per location are suggested for obtaining representative samples of the epifaunal species in most areas. Trawls equipped with odometers to record the distance travelled while on the bottom are advised for quantitative collections of epifauna (e.g., Carney and Carey, 1980).

5) Care should be taken to remove epifauna from net meshes as well as from the cod end of the net.

5.5 Treatment and Processing of Macrobenthos Samples

A scheme for the selection of appropriate methods for treatment and processing of macrobenthos samples is presented in Figure 5.9. General references for the treatment of zoobenthos samples are Thorson (1957), Birkett and McIntyre (1971), Stirn (1981) and Eleftheriou and Holme (1984). Techniques of collection and preservation of invertebrates for taxonomic purposes are described by Lincoln and Sheals (1979). A review of methods is given for zoobenthos, Section III-6, Volume 1, and for phytobenthos in Section II-5, Volume 1.

5.5.1 Recommended Practices for Treatment of Samples of Macroinfauna

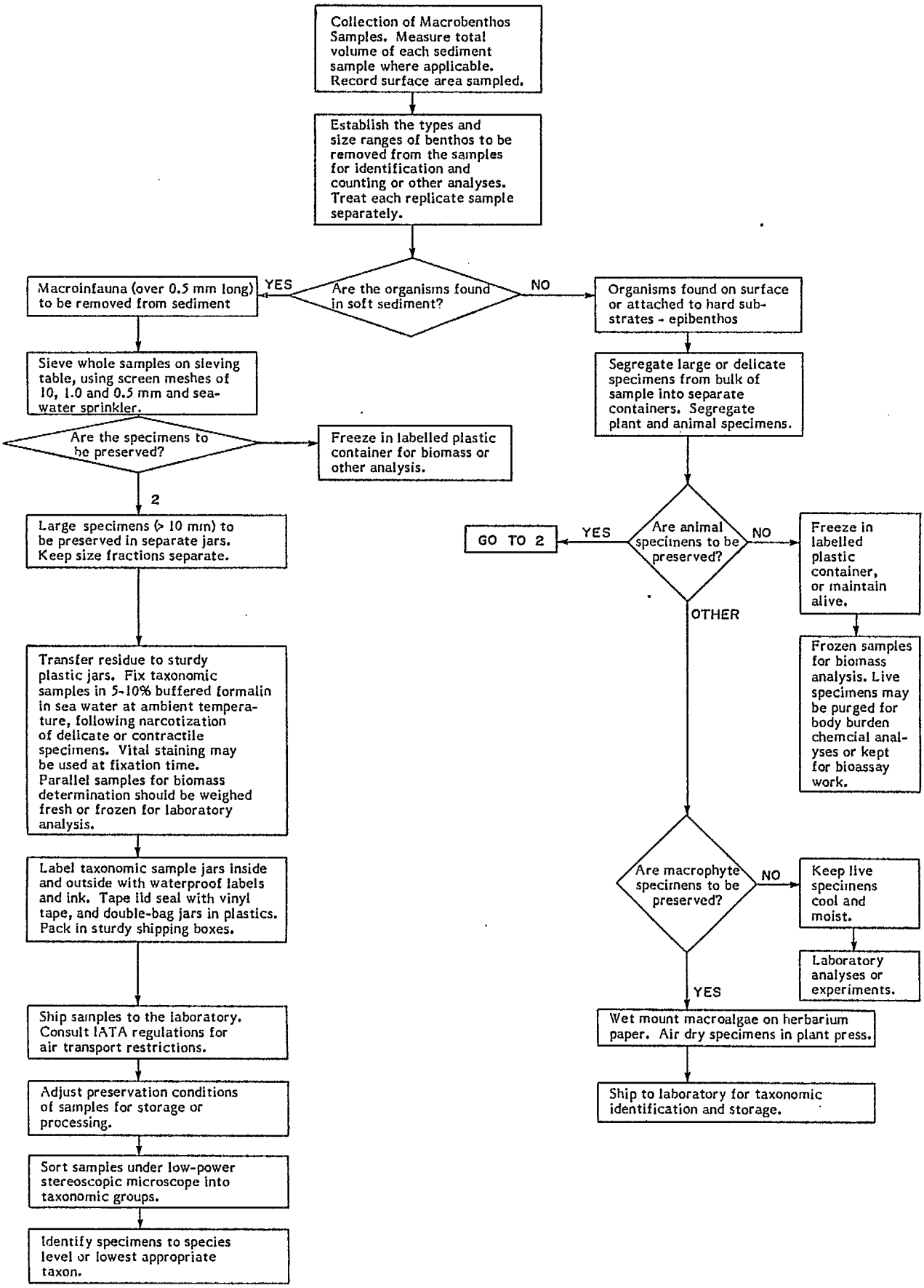
The following procedures for handling macrobenthos samples collected by grab are recommended:

1) The total volume of each sediment sample should be measured in a calibrated container. The surface area sampled should also be recorded. If subsamples are removed (e.g., by a small corer), then the surface area and volume removed should be indicated.

2) Each replicate sample should be separately sieved and fixed in the field on the day of collection (i.e., as soon as possible after sample collection). They should also be separately sorted and enumerated for species composition and abundance (see Section 5.2.3). **It is impossible to sort benthos out on the ice in cold weather unless adequate heated space is available. It is usually necessary to transport samples back to a camp. It should be remembered, however, that 50 litres of mud may weigh up to 100 Kg. The extra weight of several samples may impose transport restrictions.**

3) The extraction of macrofauna should be done (at a minimum) by sieving the whole sample on a sieving table or hopper with a 0.5 mm mesh sieve and the aid of a sea water sprinkler. A series of mesh sizes, 0.5, 1.0 and 10 mm is suggested to improve size fractionation by separating coarse mineral or detrital particles and large benthos specimens from the finer fractions. The extraction and sorting of benthos from size fractionated samples is easier and

Figure 5.9 Selection of Suitable methods for macrobenthos sample treatment and processing



more convenient than from unfractionated samples. Separation of bulky objects or specimens with complex surfaces at the time of collection or sieving will reduce some of the difficulties in subsequent sorting of the smaller organisms. The large objects removed should be inspected for small organisms on their surfaces.

4) All organisms and other residue on the screens should be transferred to suitable containers. Jars and lids made of sturdy plastic are more resilient than glass jars for shipping. Specimens for other analyses such as chemical body burden measurements should be collected in separate grab hauls and prepared according to procedures recommended for specific types of analyses (metals or organics; see for example Section 6.2.3 and 7.3.2).

5) The organisms should be fixed in a buffered solution of 5-10% formalin in sea water at ambient temperature (following narcotisation where appropriate, Section II-6.1, Volume 1). Borax, hexamine, or marble chips may be used to buffer the formalin against acidification. For thorough fixation, the volume of formalin to sample residue should be in a ratio of about 4:1, and the contents should be well mixed. Vital staining (e.g., with Rose Bengal) may be used at the time of initial fixation to aid in sorting. See Williams and Williams (1974) for details on vital staining.

6) Permanent waterproof labels made of paper with high rag content should accompany each sample or subsample in the container throughout all stages of processing, shipping and storage. The labels should include pertinent sampling information and sample identification. The labelling materials (paper, ink) should be inert so that interference with other analyses is avoided (e.g., ball-point ink interferes with hydrocarbon analyses). Labels on the jar lids and sides should be in addition to, rather than in place of, labels inside the containers. A suggested list of information for inclusion on labels is given in Section III-6.1.1, Volume 1.

7) Detailed accounts of sampling information should be recorded in a field sampling log book. Prepared log sheets with spaces for recording pertinent data and other types of observations or samples taken at the station are convenient for this purpose. This information should be retained in permanent files for later reference and analysis of data.

8) Before shipping to the laboratory, jars containing formalin should be sealed with vinyl tape and double-bagged in polyethylene plastic to prevent leakage of contents, and secured with packing in sturdy shipping boxes. **Formaldehyde is a restricted item for air transport.** Concentrated formaldehyde should be shipped by surface transport in sturdy leakproof containers. Samples containing dilute formalin may be shipped by commercial air carriers if properly packaged, labelled and declared as hazardous goods according to IATA regulations.

9) After shipment to the laboratory and a fixation period of 1 week to 1 month, the formalin-preserved specimens for taxonomic identification should be transferred to 70% ethanol or isopropanol to prevent decalcification of delicate calcareous structures and to reduce the exposure of taxonomists to formaldehyde fumes.

5.5.2 Recommended Treatment of Samples of Epibenthos

Samples of epibenthos from dredges, trawls and airlift samplers can be handled according to the procedures below if sieving is not required to separate the organisms from sediment. Samples requiring sieving can be treated in the same way as samples of macroinfauna (section 5.5.1).

1) For determination of the total wet-weight biomass of macroepifauna, the sample in the cod end of the trawl, dredge or airlift can be transferred into a tared mesh bag, drained, freed of detritus, blotted to remove surface moisture and weighed on a top-load balance (see Section III-6.2.1, Volume I).

2) The sorting, as close to species level as feasible or at least to separate plant and animal specimens, should be done in the field. The faunal component of the sample can be preserved as in Section 5.4.1. Specimens to be used for chemical body burden analyses should be treated according to the recommendations for the specific type of analysis (see for example Section 6.2.3 and 7.3.2). Representatives of each species used for various analyses should be preserved for species identification. The numbers of specimens of each species removed from a quantitative sample should be recorded to permit their inclusion in species enumeration.

3) Preservation of macroalgae for species determination is generally done by wet mounting the algal specimens on herbarium paper and air drying them between layers of wax paper and newsprint in a plant press. The layers of waxpaper and newsprint should be changed frequently in the first few hours of drying. Macrophytes to be kept alive for laboratory analyses or experiments should be kept cool and moist. A general reference on phycological methods is Stein (1973).

5.5.3 Sorting and Analysis of Macrobenthos Samples

Benthos sample sorting is reviewed in Section III-6.1.2, Volume I. Procedures which help to minimize sorting errors are given below:

1) The use of low-power stereoscopic microscopes (e.g., Wild M-5 or equivalent) and channelled counting trays with background grids (e.g., Schlieper, 1972) greatly increases the thoroughness of finding small specimens in samples.

2) The use of bulk vital staining (e.g., Rose Bengal) makes small organisms easier to distinguish from debris (Section 5.5.1, No. 5).

3) The efficiency of sorting can be checked by quality control procedures (Section III - 6.2.3, Volume I) and by examining the distribution of individuals among the species present for deviations from a log-normal distribution. As indicated by Gray and Mizra (1979) such deviations may result from the failure to find rare species or from the inaccurate identification of species.

5.6 Analysis of Macrobenthos Samples

5.6.1 Estimation of Biomass

Biomass, or standing crop, can be defined as the amount of living substance making up the population of organisms under study. Usually it refers to the weight of a sample population which was obtained from a sampled surface area or volume. For comparibility and convenience, biomass of benthos is generally reported on a per m^2 basis, and is calculated from the mean of replicate samples from a station or group of stations. The choice of units of biomass usually reflects the method for its

estimation, which can be in terms of wet weight, whole or decalcified dry weight, or ash-free dry weight, carbon and/or nitrogen content or caloric (energy) equivalent.

5.6.1.1 Wet-Weight Biomass

Wet weight is the easiest, but least reliable measurement of biomass. The following practices are advised for this approach to biomass estimation:

1) Use of unpreserved specimens is best, since preservation generally results in significant loss of organic material (see Section III-6.2.1.1, Volume I) and changes in length-weight relationships.

2) Wet weighing can be done in the field on a top-load balance unless samples are so small that an analytical balance is needed for adequate precision. Specimens should be free of sediment and detritus, and blotted to remove surface moisture. See Howmiller (1972) for an alternate laboratory method for removing surface water from small organisms by centrifugation.

3) The removal of shells and other hard parts from large specimens is not advised unless it can also be applied to smaller species or individuals. If this approach is used, though, it should be clearly stated.

4) If specimens cannot be weighed in the field, an alternative method is to collect a parallel series of samples which can be frozen and used for laboratory determination of wet (and dry) biomass.

5.6.1.2 Dry Weight

The dry biomass is the weight of completely dehydrated specimens of the sampled population. There are three main ways of removing water from the tissues:

(a) heating in an oven at 70-100°C, atmospheric pressure;
(b) desiccation under vacuum at temperatures up to 60°C;
(c) freeze drying or lyophilisation

Provided that the dry tissue is not needed for other analyses and that enough

time is allowed for complete drying, all three of these methods will give similar results. The following practices and precautions are recommended, where applicable:

1) A test run should be made to check the minimum time for complete dehydration (constant weight) before a large number of measurements is started with a given drying device. This may be necessary because larger organisms require longer to dry to constant weight than small ones.

2) If an oven is used, temperatures should not go over 100°C, or losses of volatile tissue substances may result. Samples should be cooled to room temperature in a desiccator before weighing.

3) Vacuum drying at 60°C takes a relatively short time, denatures enzymes which may cause autolysis at room temperature, and does not result in serious volatilisation losses. Specimens dried this way are also suitable for colorimetry or dry-ashing. See Crisp (1971, 1984) for further details on these procedures.

4) If dry-ashing is to be done as well, the same dishes (silicon or heat resistant crucibles) should be used for both procedures to save time.

5) Lyophilisation is the gentlest drying method, in general, and is advised if further chemical analyses are to be performed on the samples.

6) Dried samples are best stored either sealed from moisture in a deep freeze or under continuuous desiccation at room temperature.

7) Top-loading balances may be adequate for weighing large dry macrobenthos samples. For small samples, analytical balances (with ±1% reproducibility) may be used. A microbalance is needed for very small samples.

8) For all dry biomass procedures, specimens must be free of sediment or detritus and sea water. A rinse in distilled water (for up to 30 minutes) is advised prior to desiccation.

9) Calcareous exoskeletons may be removed by hand or by decalcification

treatment if the practice is applied consistently to all taxonomic groups. See Humason (1965) pp. 29-31 or Section III-6.2.1.2, Volume 1 for details on decalcification. **The ashing procedure (Section 5.6.1.3) produces more accurate biomass results than decalcification.**

10) It is critical that every drying procedure be carried out under identical conditions if seasonal surveys are conducted in a given area. This precaution prevents systematic variation in drying efficiency which may be mistakenly interpreted as a seasonal effect.

5.6.1.3 Ash-free Dry Weight

The ash content of the benthos samples, which is made up of inorganic substances such as salts, silica, calcium carbonate and phosphate, is determined after dry weighing by incinerating the organic substances in a muffle furnace. References on measuring ash weight are Crisp (1971, 1984) and Stirn (1981). **The following practices and precautions are recommended:**

1) Samples can be dry weighed in tared crucibles suitable for the ashing procedure.

2) To ensure complete removal of the organic matter, an incineration period of four to six hours at 500-600°C is advised.

3) It is desirable to use equipment with stable thermoregulation and a thermorecorder.

4) When incineration is completed, the samples can be cooled to about 80°C in the open, and then to room temperature in a desiccator, before they are weighed on an analytical balance or microbalance.

5) Ash-free dry weight is calculated by subtracting the ash weight from dry weight. It is a reliable estimate of total organic matter for samples containing mainly soft-bodied organisms.

6) For samples with a high proportion of organisms with calcareous exo-skeletons, the ash content is seriously underestimated by the above procedure

due to significant volatilisation of inorganic carbon dioxide from carbonates and hydrated water from silicates. Therefore, groups such as molluscs, barnacles and echinoderms should be treated differently in order that the dry organic weight of the soft tissues and exoskeleton can be measured separately. Crisp (1971) has described techniques appropriate for the various groups involved.

5.6.1.4 Estimation of Biomass in Terms of Nitrogen Content

An alternative to estimating biomass as ash-free dry weight is to chemically analyse the organic tissue for nitrogen content, which is assumed to be proportional to the amount of living matter. A review of methods based on nitrogen analysis is presented by Giese (1967). Details of the classical Kjeldahl measurement of nitrogen are given by Barnes (1959. pp. 148-150). It is recommended that results obtained by this procedure be reported as biomass (mg Kjeldahl nitrogen) rather than as biomass (mg total nitrogen).

5.6.1.5 Measurement of Caloric Content

The caloric content of any substance is defined as the energy released during complete combustion of one gram of the dry substance. The caloric content of a dry tissue homogenate from a whole organism is an important factor in biological production studies for converting dry biomass into energy units. Seasonal changes in caloric content must be allowed for when converting dry weight into energy, due to changes in energy content between life-history stages of many organisms.

References on detailed procedures for measuring caloric content are Phillipson (1964) for calorimetry and Hughes (1970) for the iodate oxidation method. Crisp (1971, 1984) has reviewed calorimetry and chemical methods of measuring caloric content of organic matter.

5.6.2 Taxonomic Identification and Enumeration

The analysis of benthic communities is based on accurate identification and enumeration of specimens in benthos samples. A comprehensive study of benthos requires a team of good taxonomists for the identification of the major taxonomic classes, and access to outside expertise for assistance in identification of the

remaining biota. A marine benthic research team should include specialists for the important taxonomic groups such as polychaetes, crustaceans (e.g. amphipods, isopods, and cumaceans), molluscs and echinoderms. Some groups which are usually represented by a small number of easily recognizable species, can be identified by non-specialists. Outside specialists may be needed for taxa which may be unfamiliar to the members of the team, such as bryozoans, sponges and ascidians. Mayr (1969) and Gosner (1971) have described the types of taxonomic keys and have provided introductions to the practices of systematics and taxonomic identification.

Specimens of the major taxonomic groups may be identified to species or temporarily coded in situations where a species is recognized but the positive identification needs further work. The abundance of all species present in the sample can be counted and recorded either during the identification process or afterwards. Only specimens which were viable at sampling should be included, not skeletal or other remains. In the case of damaged specimens, only the head segments are counted as specimens, not appendages or other body parts. References used in the identification of specimens should be cited, at least in the initial reporting of results.

5.7 Quality Control in Benthos Studies

The purpose of a quality assurance program for a measurement system is to reduce errors to tolerable limits and to ensure that the measurements are likely to be of acceptable quality (Taylor, 1981). Quality control is the mechanism within a quality assurance program which controls errors, while quality assessment is the means to verify that the system is operating within acceptable limits. Since quality is subjective and varies according to situation, tolerable limits of error must be determined on the basis of cost-benefit considerations for each property to be measured. Quality control in benthos studies is complicated by the fact that true values of parameters such as species density and biomass for natural populations are unknown. Therefore, it is impossible to determine the "accuracy" of the methods used (i.e. how close the estimates are to the true values). Precision (the relative difference between replicate or repetitive measurements) of benthos estimates can be established, however.

Measures for quality control which are applicable to benthos studies (e.g. Stirn, 1981; Tetra Tech, 1982) include:

1) Good laboratory practice, which requires that:

   a) proper procedural protocols are written and observed in practice. These include sampling protocol, steps for quality control in the field, methods of sample treatment, methods of analysis, quality control steps in the laboratory and standardized calculation and data reporting;
   b) high standards of personnel training and laboratory management are used; and
   c) field and laboratory procedures are inspected and audited by a third party.

2) Field sampling quality control including:

   a) collection of an adequate number of samples;
   b) replication to the extent possible of a fixed percentage of samples;
   c) preparation of detailed written sampling directions;
   d) documentation of sampling in field log books;
   e) verification of the number, type and location of samples obtained;
   f) careful selection, use and maintenance of sampling equipment;
   g) adequate inspections to guard against inadequate sample preservation, leakage or loss during collection, transfer, shipment and storage;
   h) adequate chain of custody and labelling for samples; and
   i) collection of reference (background) and contingency samples.

3) Laboratory quality control:

   a) preparation of detailed written protocols for sorting and identification;
   b) a fixed percentage (e.g. 5%) of all samples should be resorted by individuals different from those who conducted the original sorting. This should be a double-blind test. Results of the second sorting should be maintained.
   c) a fixed percentage (e.g. 5%) of the species identified by each member of the taxonomic team should be verified by a competent independent taxonomist. Results of the verifications should be maintained.

d) complete records or log sheets of sorting, processing and laboratory analysis of each replicate sample should be maintained in a file with sampling data.

e) a voucher or laboratory museum collection of specimens which are representative of each species (or lowest taxon) collected during benthos surveys in a study area should be prepared and maintained. Efforts should be made to have the identifications verified and representative specimens deposited with a public museum collection whenever possible (e.g. National Museum of Natural Sciences). Contacts in the National Museum of Natural Sciences, Ottawa, Ontario, K1A 0M8 are listed in the Directory of Marine and Freshwater Scientists in Canada; Anon (1984). The laboratory museum collection should be maintained in 70% ethanol or isopropanol according to appropriate procedures (Levi, 1966). This collection is invaluable for reference and verification purposes.

f) comprehensive and updated taxonomic literature should be maintained in the laboratory for reference during the identification process.

g) inter-laboratory comparison studies may be conducted to establish or verify the level of performance of a laboratory or taxonomist and to provide a comparison of proficiencies of participating laboratories. For example, Ellis and Cross (1981) have described a protocol for inter-laboratory comparisons of biological species identifications or "Ring tests".

Since precise taxonomic identifications are necessary for reliable analyses of communities, it is recommended that a skilled team of taxonomists and collaborating specialists be employed, and appropriate guidelines for quality control observed.

6. METHODS FOR THE ANALYSIS OF METALS IN SEDIMENT, TISSUES AND INTERSTITIAL WATER

6.1 Introduction

The purpose of this section is to provide assistance in choosing methods to study metal concentrations. It is based on the information generated by the Review of Methods (Volume I). The present chapter attempts to develop guidelines for selecting those methods which are appropriate for specific purposes.

For example, an investigator may wish to accurately determine background metal concentrations, anomalous concentrations or those above legally specified limits, e.g. the O.D.C.A. limits for Hg and Cd. An environmental assessment may require the determination of only two particular elements or a broad scan of what can be detected. The objective may be to measure the total metal concentration, some fraction thereof (e.g., the "biologically available" fraction, or some particular metal species (e.g. methylmercury). The number of samples to be analysed may be large or small, and the accuracy and precision required is variable.

Three types of environmental samples are considered:

1. **sediment interstitial waters,**
2. **whole sediments, and**
3. **benthic organisms.**

The analysis of these samples for metals follows the general outline - sampling and pretreatment, digestion, preconcentration and instrumental quantification. Sediments and tissues must be digested to produce a solution that is suitable for instrumental quantification by these techniques; digestion is not essential for interstitial waters. The various factors to consider are outlined in Figure 6.1.

Three instrumental techniques are considered:

1. **flame atomic absorption spectrometry (F-AAS),**
2. **graphite furnace atomic absorption spectrometry (GF-AAS)** and
3. **inductively coupled plasma atomic emission spectrometry (ICP-AES).**

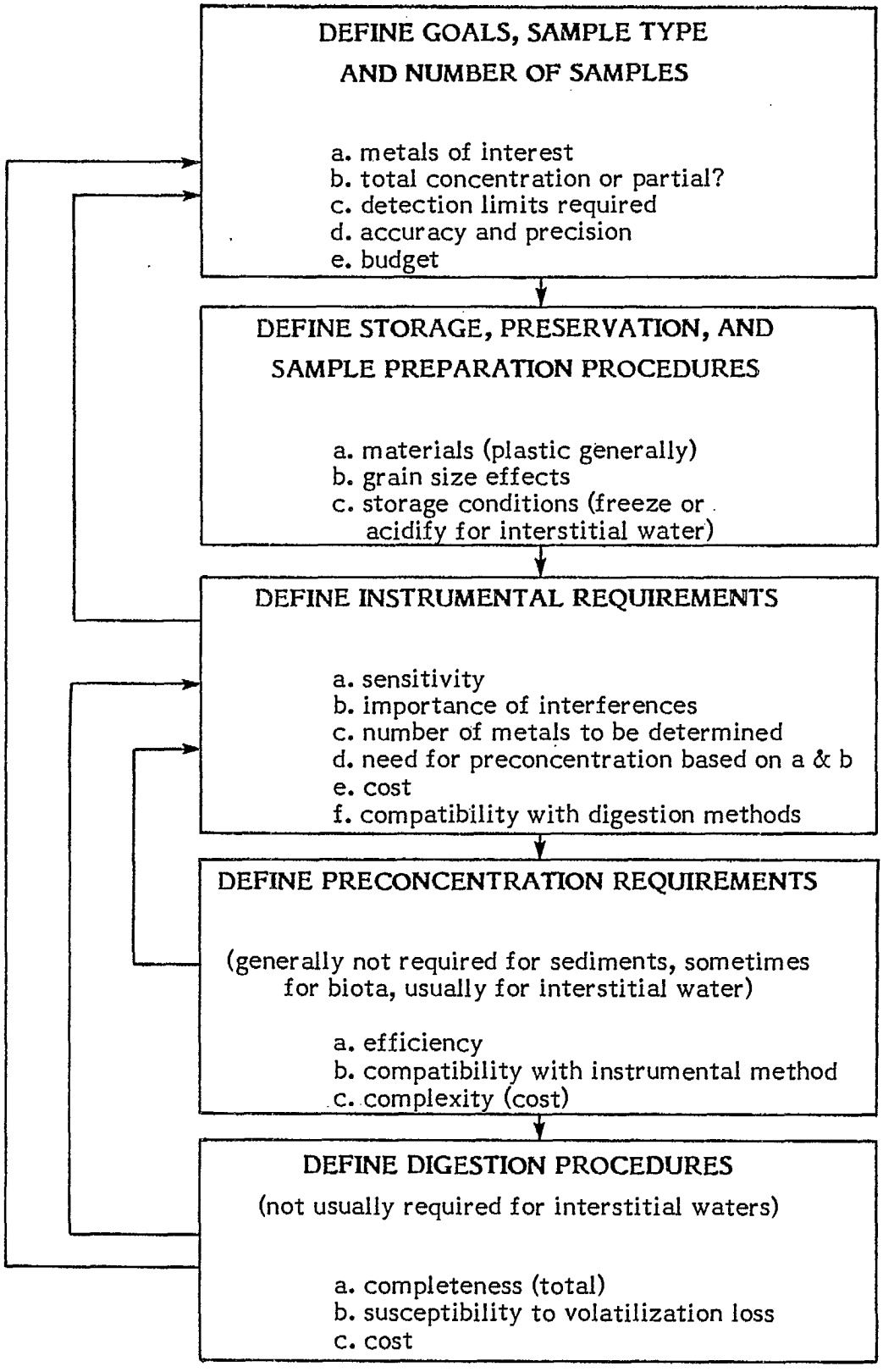


Figure 6.1 Outline of Factors to Consider in Choosing a Method of Analysis for Metals in Sediments, Biota or Interstitial Waters.

Because the instrumental techniques have different sensitivities and the sample types have different concentration levels, the type of sample to be analyzed affects the choice of instrumental technique. Flame AAS and ICP do not have sufficient sensitivity to analyse interstitial waters without preconcentration, while GF-AAS can sometimes analyse interstitial waters without preconcentration. Sediments and tissues are usually analysed by F-AAS and ICP, reserving GF-AAS for the more difficult metals. Sample type also affects the need for preconcentration. Preconcentration is almost ubiquitous for interstitial waters, common for tissues and rare for sediments.

Sample type determines the method of digestion that is employed. Complete dissolution of sediments requires HF plus oxidizing acids. Alternatively, alkaline fusion with acid dissolution of the flux can be used. Tissues require a more oxidizing mixture of acids and HF is usually not needed. Alternatively, tissues can be first ashed in a high or a low temperature (atomic oxygen) furnace. Interstitial waters are not digested unless it is thought that metal-organic complexes or organometallic compounds might interfere with the preconcentration. Digestion of interstitial waters can usually be accomplished under milder conditions than those needed for tissues.

The metals differ in their chemical properties and this affects the choice of digestion and preconcentration methods. The metals also differ in their detection limits on a given instrument. Rather than develop individual methods for each metal, chemists usually try to devise general methods for groups of metals to take advantage of similarities in behaviour. Multi-element methods often result in a considerable time savings. **For convenience, the following metals are considered as a single group: Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn. These elements will be referred to as Group I metals in the text that follows.** Many of the methods for metals of this group are multielement methods which include three or more elements.

Arsenic and mercury are special cases because of their volatility, their formation of organometallic compounds and their extremely low concentrations. Also, special preconcentration techniques are available for them which are superior to other techniques in sensitivity and freedom from interferences. Mercury is unique in that it is nearly always determined alone by cold vapour atomic absorption spectrometry (CV-AAS). The technique involves reduction of mercuric ions to elemental mercury which is separated from the solution as a vapour. For arsenic, the hydride generation (HG) technique is similarly important. Arsenic can also be determined in combination with other hydride forming elements by HG-AAS or HG-ICP.

To facilitate comparison and evaluation the methods have been broken down into their component parts. In preparing a plan it is usually necessary to consider these factors in this order and this is how they have been presented in this Guide.

1. **sampling and pretreatment.**
2. **instrumental quantification,**
3. **preconcentration,**
4. **digestion,**

The two general criteria that have been used to evaluate the methods are performance and cost. Assessing the level of performance and cost of the methods being compared is usually not easy. Performance can be measured by the limit of detection, accuracy and precision. The quality and quantity of information given by methods reports on these quantities varies. There are very few experimental studies where different methods have been objectively compared. Specific information on costs is generally lacking in methods reports, but the factors affecting cost (i.e., capital costs, sample throughput and level of skill required) can usually be assessed. Therefore, due to the limited nature of the information available from the methods reports, the comparison and evaluation of the methods will necessarily be somewhat subjective.

6.2 Sample Preparation and Sample Preservation

6.2.1 Sampling and Sample Preparation Methods for Interstitial Waters

Due to the extremely low concentrations of trace metals, the sampling and storage of interstitial water can be very problematical from the standpoint of contamination. Special techniques that are required for seawater analysis, such as the use of clean room facilities, special purification procedures for container surfaces and ultra-pure reagents should be applied equally to interstitial waters. These techniques have been discussed by Batley and Gardner (1977) and Erickson (1977). Also, techniques that are applicable to specific metals are usually given in papers describing the analytical methods for those metals (e.g. Bloom and Crecelius (1983) for mercury, Andreae (1977) for arsenic). In addition, careful attention must be given to the procedures used to collect sediment samples and to extract interstitial water from them, not only to avoid contamination but also to avoid

creating other artifacts. For example, changes in concentration or speciation might result from exposure to air or temperature change before or during extraction of interstitial water from sediments. The safest procedures are those which extract and preserve samples in conditions as close as possible to the natural ones. The paper by Kruikov and Manheim (1982) discusses the problems involved in sampling interstitial waters.

There are two commonly used techniques for extracting interstitial water from sediments - centrifugation and low pressure squeezing. Both techniques require that a fresh, representative, uncontaminated sample of whole sediments be obtained, usually by grab or core sampler (Section 3.2). The in situ extraction techniques, though they should in principle subject the sediment-water system to less perturbation, are usually impractical in the Arctic and are therefore excluded from this treatment. Batley and Giles (1980) tested three centrifuge techniques and low pressure squeezing for efficiency. For sandy sediments, solvent displacement centrifugation gave the highest recovery; while for clay sediments, low pressure squeezing and solvent displacement centrifugation were about equally effective. The solvent displacement centrifugation technique described by Batley and Giles (1980) utilizes a heavy, water immiscible solvent, 'Fluoriner FC-78', to displace interstitial water from the sediments during centrifugation. The low pressure squeezing apparatus described by Presley et al.(1967) is suitable for trace metal work.

A low pressure squeezer may be able to produce a larger quantity of interstitial water in less time than centrifugation, but centrifuges are more common than low pressure squeezers. Both techniques can be applied on board a research vessel. On the average, it appears that the low pressure squeezing device of Presley et al. (1967) and the centrifuge methods of Batley and Giles (1980) are about equally suitable for extracting interstitial water samples from arctic sediments for trace metal determinations.

6.2.2 Sample Preparation Methods for Sediments

The procedures used for sample storage and preparation for analysis depend on the kind of determination that is to be performed. Figure 6.2 outlines typical sequences for the main kinds of determination. After the grab or core sampler has been retrieved, a representative subsample is obtained with a suitable implement (e.g. a plastic scoop or coring tube), and this is placed in a labelled container for transport to the laboratory. Whirlpak bags and wide-mouth jars (glass or plastic) are

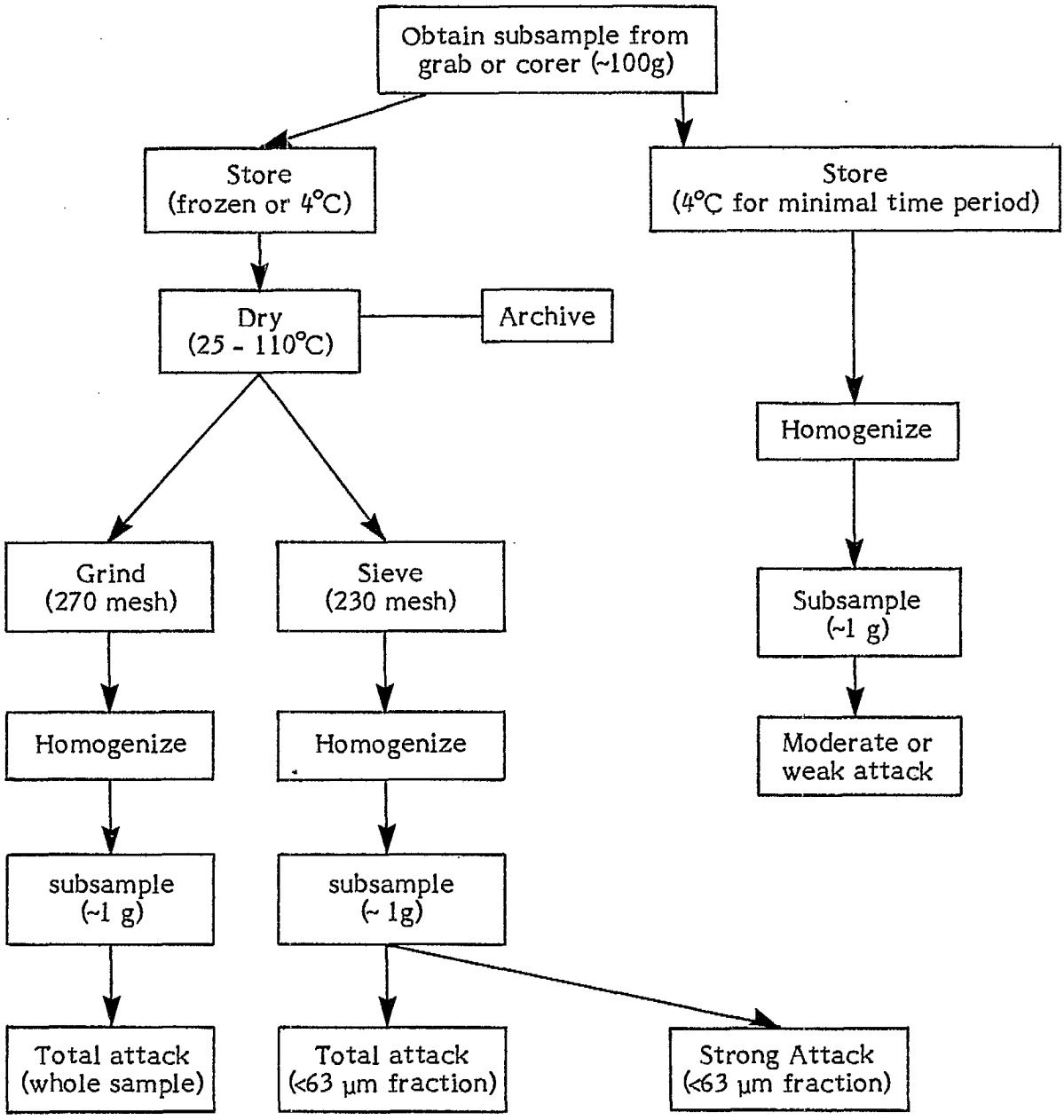


Figure 6.2. Sample preparation sequence for sediments.

usually employed for temporary storage. These should be clean, but special acid cleaning procedures are probably not necessary. Containers should be tested for potential contamination effects. Direct contact of the wet sediment sample with metal surfaces should be avoided. The samples should be stored frozen or at a sufficiently low temperature (4°C) to limit biological activity. For long-term storage (archiving), samples should be stored dry. It is recommended that sediment samples be dried in the laboratory before they are analyzed. Dried sediments are easier to sieve, grind and homogenize than are wet sediments. Metal concentrations are usually expressed on a dry weight basis, so drying of a separate subsample is necessary if wet sediments are to be analyzed. For determining most metals, drying at temperatures from 25-100°C, can be recommended. Freeze drying may be preferred to air or oven drying because it results in a powdery material; air or oven drying of clay sediments results in hard aggregates. **Mercury is sensitive to drying; oven drying should therefore be limited to a temperature of 40°C.** Air drying and freeze drying can also be recommended for Hg.

In order to obtain a representative small subsample (~1 g) for digestion, the sample should be reasonably homogeneous. Grinding of dry sediments to a uniform particle size facilitates homogenization as does screening to remove particles larger than say 63 μm. Homogenization of wet sediments can be effected with a plastic spatula or else by kneading the sample in a plastic bag.

Grinding of sediments also increases the surface area and promotes more rapid dissolution. Grinding is recommended for total attack methods, but is not recommended for strong attack methods because precision and comparability may be degraded. Grinding of dry sediments can be accomplished without contamination using an agate mortar and pestle. Sieving of dry sediments to ca. 230 mesh (63 μm) is recommended for removing the often dominating effect of grain size on trace metal distributions (Forstner and Wittman, 1979; Salomons and Forstner 1984). Sieving is obviously not appropriate when an analysis of whole sediments is required, but is particularly appropriate when a strong attack is used to survey anthropogenic inputs. Nylon sieves are recommended to avoid contamination. When moderate or weak attack methods are used, or when particular species are determined, it is recommended that whole, wet sediments are extracted as soon as possible after collection; drying, sieving and grinding tend to introduce undesirable artifacts.

6.2.3 Sample Preparation Methods for Tissues

Methods for preparing biological samples for trace metal determination must ensure that the sample is representative of the tissue or organism that is being studied and that its composition has not been altered by handling or storage. After the organisms have been retrieved, they should be washed with clean seawater to remove sediments and adventitious contamination. Depuration of clams and mussels is optional; it is recommended whenever there is a concern that food or sediments inside the gut may vitiate the results. The organisms can be stored frozen in plastic bags or glass or plastic jars. These should be very clean but acid purification is probably not necessary. To avoid necrochemical artifacts the organ or tissue that is to be analyzed should be removed immediately after sampling. If that is not possible the whole organism should be analyzed (minus the shell in the case of bivalves). Freeze drying of the tissue sample is recommended for long term storage.

As all tissue samples will be heterogeneous, they should be homogenized before subsampling. This can be accomplished by mincing, chopping or blending the wet sample, either by hand or by machine. Care should be taken to choose the right implement at this stage as some may contaminate the sample with the metals being analyzed. For example, stainless steel homogenizers (such as Polytron or UltraTurrax) may be safe for arsenic and mercury, but are definitely not safe for Fe, Cu, Ni and Cr. Alternatively, the tissue sample can be homogenized after drying by grinding in an agate mortar and pestle. Drying also facilitates long term storage. For elements other than mercury, oven drying at up to 100°C is recommended. When mercury or methylmercury is being determined, only freeze drying can be recommended. To be entirely safe, non-metallic tools and labware should be used throughout. After homogenization and subsampling, the wet or dry tissue sample is digested by wet or dry ashing techniques. If it is digested wet, a separate subsample should be dried to determine the moisture content, since concentrations are usually expressed on a dry weight basis.

6.3 Instrumental Methods

6.3.1 Factors Affecting the Choice of Instrument

Usually the first decision to make is which instrument to use. The choice depends on which are sensitive enough to determine the metal concentrations in the

samples. The final metal concentrations in the solutions presented to the instrument should be within the linear operating range of the instrument. Whether to employ a preconcentration step depends on whether the metal concentrations after digestion are below the limits of quantification and whether other elements in the sample matrix interfere strongly with the elements being determined. Thus the decision of which instrument to use, and whether to employ preconcentration interact. The kind of digestion that is appropriate also sometimes depends on the choice of instrument. Flame AAS, for example, can tolerate more dissolved solids and viscosity than can ICP and GF-AAS. Hydrofluoric acid is incompatible with the nebulizer systems commonly used with AAS and ICP. Sulfuric acid can produce an undesirable amount of background absorption with GF-AAS.

Instrumental sensitivity decreases in the order, GF-AAS $\gg$ ICP > F-AAS, whereas the metal concentrations generally increase in the order, interstitial water $\ll$ tissues < sediments. This relationship explains why interstitial waters are analysed almost exclusively by GF-AAS after preconcentration and why sediments are usually analysed by F-AAS or ICP. GF-AAS is too sensitive to determine most elements in sediments and is therefore usually reserved for those elements for which F-AAS and ICP are not sensitive enough.

To estimate whether the final solution concentrations will be within the operating ranges of the instruments requires some assumptions. The sample concentrations must first be assumed. These are given in Tables III-2.3 (interstitial waters), III-2.8 (sediments) and III-2.17 (biota) of Volume I. Sample concentrations, together with the dilution factor (for the digestion) and the pre-concentration factor, determine the final solution concentrations. Typical values for these factors are assumed; sometimes different combinations are explored. It is assumed that the method limit of quantification, L.O.Q., is equal to ten times the instrumental limit of detection, L.O.D., which are given in Table III-2.1 of Volume I. The upper limit of the linear operating range is assumed equal to 10^3 x L.O.D. for flame and GF-AAS and 10^5 x L.O.D. for ICP.

The procedure used for estimating whether the final solution concentrations will be within the linear operating range of the instrument consists of:

1) calculating the final solution concentration range from the original sample concentration range and the typical dilution and preconcentration factors, and,

2) comparing this with the linear operating range of each instrument for each metal.

The results are then presented in a table in which the metals are divided into four categories:

1) **too low** - sample concentration range is completely below the operating range of the instrument;

2) **marginal** - sample concentration range is partly below the lower limit of the operating range;

3) **within** - sample concentration range is within the operating range;

4) **too high** - sample concentration range is partly or completely above the upper limit of the operating range.

Finally, the results are checked against the published method detection limits or limits of determination quoted in Volume 1. Method detection limits are not always defined in the same way. The exact definitions are sometimes but not always given in the papers describing the methods. In general, the method detection limits correspond roughly to the criterion used here, i.e. 10 x L.O.D. (instrumental), and the two sets of results are consistent.

After determining which instruments are suitable from the standpoint of sensitivity, other considerations come into play in making a choice of instrumental method. Accuracy and precision can be assessed, since specific information on these parameters is usually given in method reports. Accuracy equals absence of specific interferences and is usually proven by analysis of standard reference materials (SRM's). Of the three instrumental techniques, graphite furnace is generally the most subject to interference and, after automatic corrections for spectral interference, ICP is generally the least. Since different kinds of interference affect F-AAS and ICP, the two instruments could be used synergistically to check the accuracy of instrumental quantification. The overall precision of the determination is ultimately limited by the instrumental precision. Usually however, the overall precision does not approach this limit very closely. Also, the instrumental precisions are not widely different, so it is assumed that the instruments are equivalent in this respect.

Although specific cost information is not usually given in method reports, the factors affecting cost can be estimated. Instrumental costs decrease in the order ICP > GF-AAS > F-AAS. Sample throughput for F-AAS is greater than for GF-AAS. ICP has a potentially much greater throughout than F-AAS since a large number (20-30) of elements can be determined simultaneously. Whether a preconcentration step is necessary will affect the analysis time. In terms of operational difficulty, ICP is difficult to learn but easy to operate routinely. Flame AAS is easy to learn and to operate. GF-AAS is moderately difficult to learn and to operate. In terms of availability, F-AAS > GF-AAS = ICP. Taking all factors into consideration, the cost per element is expected to be somewhat greater for GF-AAS than for F-AAS. Depending on the number of elements to be determined, ICP can be more expensive than F-AAS (for fewer than about 3-5 elements) or cheaper (for more than that number). Commercial laboratories usually charge a fixed amount for an ICP determination, which quantifies 20-30 elements. Therefore, the cost per element needed is inversely proportional to the number of elements, and ICP would be very much cheaper than AAS if values for 20 - 30 elements were needed. It is unusual however, for a multielement method to give accurate values for that many elements.

It may be advantageous to use two or more instruments for determining a particular combination of metals in the same solutions. For example, rather than use GF-AAS for Cr, Cu, Cd, Ni and Pb in sediment solutions, it would be cheaper and probably more accurate to use F-AAS for Cr, Cu and Ni, and to use GF-AAS for Pb and Cd, which require GF sensitivity. In most cases, the same sample solutions can be quantified by different instrumental techniques; when a large number of elements must be determined, ICP has a big cost advantage over F-AAS, and would therefore be preferred, all other things being equal.

6.3.2 Interstitial Waters

6.3.2.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

The analysis of seawater and interstitial water for Group I metals places high demands on the sensitivity of the instrument. There is little published data on trace metal concentrations in interstitial waters. In assessing the suitability of different instrumental techniques, it has been assumed that they vary from normal seawater levels to those that are hundreds of times greater. Theoretical L.O.Q.'s were not calculated. At such low concentrations they may be more dependent on the blank

than on the instrumental sensitivity. Instead, the method L.O.D.'s or procedural blanks quoted in Volume 1 were directly compared with the normal seawater concentration ranges given in Table III-2.3, Volume I. From these comparisions, it is clear that sensitivity decreased in the order: GF-AAS (with preconcentration) > GF-AAS (without preconcentration) $\gtrsim$ ICP (with preconcentration). While GF-AAS with preconcentration is sufficiently sensitive to determine most of the metals at the lower ends of their concentration ranges, the latter two techniques are not. Flame AAS with preconcentration is even poorer, and for this reason it is vary rarely used.

The method L.O.D.'s depend on the sensitivity of the instrument, the preconcentration factor and the magnitude of the blank. To obtain a very high preconcentation factor, e.g. 500, it is necessary to extract a large volume of seawater, 500 - 1,000 mL. A 500 - 1,000 mL sample of interstitial water is impractical however, since interstitial water samplers (pressure squeezers and centrifuge) generally yield volumes in the range of 10 - 100 ml. It may be possible to reduce the volume needed for ICP quantification by employing a microsampling technique similar to the one described by Uchida et al. (1981). The successful application of this technique to interstitial waters would be a significant advance. On the other hand, preconcentration factors of 50 -200 are sufficient for GF-AAS and a final sample volume of 0.5 - 1 ml is adequate for doing a suite of five trace metals, which is a typical yield for a preconcentration. Thus the superior absolute sensitivity of GF-AAS is a very significant advantage in the analysis of interstitial waters.

RECOMMENDATION

On the basis of sensitivity, GF-AAS is the most appropriate choice. Although it may be possible to get by with a direct GF method for some metals in some samples, preconcentration will probably be necessary in most instances. The preconcentration/GF-AAS methods can be scaled down to the smaller sample volumes typically available for interstitial waters. The preconcentration/ICP methods will probably be generally less useful due to the larger sample volumes required, although the ability to analyse a large number of elements simultaneously is a compensating advantage.

Assuming that the instrumental costs would be similar for the analysis of digested sediment or tissue samples, then there would be a significant cost advantage for multielement analysis utilizing ICP. However, adapting an ICP method to the smaller sample volumes available may entail an unacceptable sacrifice to the

detection limits. Therefore ICP will not always be preferred when more than 3-5 elements are to be determined.

Direct GF-AAS methods may be somewhat cheaper than preconcentration/GF-AAS methods, but they are not sensitive enough to determine the lowest expected concentrations. Direct GF-AAS methods also suffer from the following disadvantages: they are more prone to interferences; they are equipment dependent, and they are more difficult to apply. The preconcentration methods do not have these disadvantages. Solvent extraction, the most popular technique, is fairly rapid, and it effectively removes the seawater matrix, making the instrumental determination much easier and faster. Starting with a volume of 10 -100 ml, the final volume is quite adequate and the final concentrations are nearly optimum for the graphite furnace. When three or more metals are preconcentrated at once, the total analysis time would probably not be greater than when using direct GF methods.

A possible disadvantage of preconcentration methods is incomplete recovery of metals bound to colloidal organic matter. This problem can be circumvented by employing a sample digestion method, as described in section 6.5.2.

6.3.2.2 Instrumental Methods for Arsenic in Interstitial Waters

For the determination of As in interstitial waters, arsine generation is the only technique which is sensitive enough to measure As in seawater and interstitial water. Although arsine can be quantified by gas chromatography (with electron capture detection), DC plasma atomic emission spectrometry or ICP-AES, the most popular and sensitive detection system is a quartz cuvette burner in the light path of an AAS. The use of a cold trap to condense arsine and organoarsine compounds purged from a sample combined with electrical heating to selectively volatilize the compounds increases sensitivity and enables the determination of four arsenic species in sea water, arsenate, arsenite, monomethylarsonic acid and dimethylarsenic acid. The alternatives to hydride generation AAS is graphite furnace AAS after preconcentration by ion exchange or solvent extraction. These techniques are less sensitive, more time consuming and more restricted in the species that can be determined.

The cold trap/HG-AAS method of Andreae (1977, 1983) has the best combination of desirable features for determining arsenic species in seawater and interstitial waters. It has been extensively applied and meaningful results have been

obtained. The detection limits are excellent (0.3 ng/L), the analysis time is 30 minutes and only 10-100 mL of sample is required. Interferences are minimal and contamination is not as big a problem as with some other elements, e.g., mercury. Samples can be preserved by quick-freezing them without altering the species ratios. The apparatus is specialized but not difficult or expensive to fabricate. Commercial systems are also available (e.g., from Varian and Perkin-Elmer).

6.3.2.3 Instrumental Methods for Hg in Interstitial Waters

a) Total Mercury

The mercury vapour technique, in which mercuric ions are reduced to elemental mercury and partitioned into the gas phase, must be used to determine Hg in seawater and interstitial waters. A quartz cuvette in the light path of an atomic absorption spectrometer is the most common detection system; atomic fluorescence and DC plasma emission spectrometry are considerably rarer. The other common preconcentration instrumental techniques do not have sufficient sensitivity. The sensitivity of the mercury vapour technique is considerably improved and potential interferences are reduced when a gold trap is placed between the sample reduction flask and the optical cell of the AAS. A gold trap consists of a quartz tube containing gold foil or gold-coated glass beads; gold amalgamates the Hg vapour swept from the reduction flask. When the trap is heated by a coil of resistance wire, Hg is quantitatively released and transferred by a stream of argon or nitrogen gas to the detector.

The method of Olafsson (1983) typifies the gold trap CV-AAS technique of determining mercury in sea water. The lower limit of quantitative determination is about 2.5 ng/L. The two stage gold trap CV-AAS method of Bloom and Crecelius (1983) has the ability to determine Hg in sea water down to 0.1 ng/L. The accuracy and precision was demonstrated by a collaborative study and by analysis of SRM's. Advances in the use of extremely clean reagents and special sample handling techniques are partly responsible for achieving the extremely low detection limit. **The methods of Olafsson (1983) and of Bloom and Crecelius (1983) are well proven and should not present any special difficulty in their application to interstitial water.**

The automated CV-AAS method of Goulden and Anthony (1980) presents an interesting alternative for measuring mercury and its species in interstitial waters.

Through a selective reduction scheme, the method is able to determine alkyl, aryl and inorganic mercury. The detection limit, 1 ng/L, is achieved through efficient partitioning of elemental Hg into the gaseous phase. The advantages of requiring less attention and having greater throughput and precision than the manual methods are offset somewhat by the greater initial cost and difficulty of setting up the apparatus.

b) Methylmercury

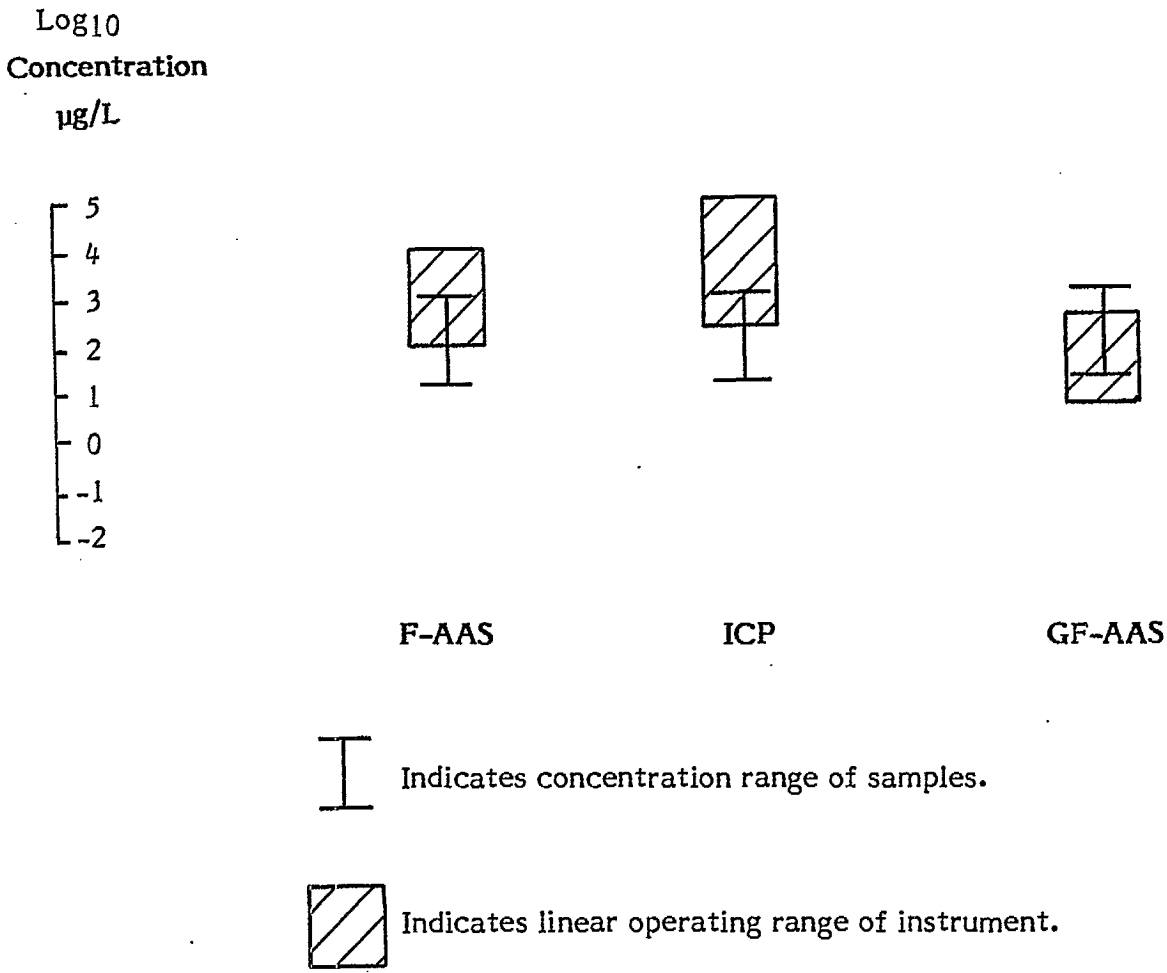
No thoroughly satisfactory methods have been found for determining methylmercury in interstitial water. Goulden and Anthony's (1980) method will determine methylmercury by difference to 5 ng/L. The sensitivity of this method could be extended by combining the selective reduction technique with the manual gold trap CV-AAS technique. A more selective direct method could presumably be derived from the benzene extraction/GC-EC methods for biota described in Section 6.4.4.2.

6.3.3 Instrumental Methods for Sediments and Biota

6.3.3.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

a) Sediments

In determining which instruments have sufficient, but not too much, sensitivity for determining metals in Arctic sediments, the concentration ranges of Table III-2.8, Volume I were used. The dilution factors arising from digestion were taken to be 100 for F-AAS, GF-AAS and ICP. The general procedure has been described in section 6.3.1. To illustrate, results for Pb are given below.



The results show that F-AAS and ICP would not be effective choices. Lead can be quantified by GF-AAS at 100 fold dilution, however. The results for Group I elements plus As and Hg are summarized in the table below.

	F-AAS	ICP	GF-AAS
Too low	As, Be, Hg	Cd, Hg, As	Hg
Marginal	Cd, Pb	Pb	
Within	Ba, Cr, Cu, Ni, V	Ba, Be, Cr, Cu, Ni, V, Zn	As, Be, Cd
Too High	Zn		Ba, Cr, Cu, Ni, Pb, V, Zn

It can be concluded that GF-AAS is indispensable for a few elements, but generally too sensitive for most. F-AAS and ICP are generally the most useful instruments in that they both can quantify the majority of metals. GF-AAS can be used to complement either F-AAS or ICP. These results are believed to be fairly

general but some of the details might change if different sample concentrations, instrumental detection limits or preconcentration factors are used. Therefore, it is recommended that the exercise be repeated using more appropriate data when they are available.

The choice between ICP and F-AAS is not restricted by sensitivity. The cost of analysis by ICP is likely to be much lower than by F-AAS when a large number of elements are determined. In commercial laboratories, the determination of 20-30 elements by ICP costs about the same as the determination of 3-5 elements by F-AAS.

Typically, GF-AAS costs a few dollars more per element than F-AAS. Where special techniques are used, such as the L'Vov platform, the price difference will be greater. These conclusions are based on the prices charged by four commercial laboratories shown in Table III - 2.12, Volume I. From the evidence presented in Volume 1, the overall accuracy and precision of the determination of metals in sediments by ICP is at least as good as that accomplished by F-AAS. The instrumental precision of F-AAS is slightly better than GF-AAS, although the overall method precision is not only limited by the instrumental precision. In comparing flame and graphite furnace for accuracy in the analysis of sediment digests, interferences are generally more likely to be encountered with the graphite furnace, but for the methods in question, the interferences have been mitigated by using standard additions or matrix modifiers. Matrix modifiers are also used in the flame methods. In the case of ICP, interferences are mainly spectral in character and are effectively corrected by spectral analysis and calculation. Since method accuracy is normally proven by analysis of reference materials, it can be concluded that serious interferences are absent. The instruments therefore appear to be on an equal footing with respect to accuracy.

RECOMMENDATION

F-AAS and ICP are the methods of choice to determine most metals in sediments. GF-AAS will probably be needed only for As, Cd, Pb and Be. Arsenic can be done with better accuracy by hydride generation AAS. Mercury must be done by cold vapour AAS. ICP is preferred to F-AAS when more than 3-5 elements are determined, because ICP is less expensive than F-AAS and just as precise and accurate. An ICP determination gives the concentration of 20-30 elements, so it is

sometimes used to screen samples for possible anomalies. Flame AAS is cheaper when less than 3-5 elements are to be determined. Flame AAS and ICP are complementary in the sense that they have different kinds of interferences.

The instrumental methods of McLaren et al. (1981) Floyd et al. (1980) and McQuaker et al. (1979) can be recommended for ICP. The methods employ different instruments, sequential and simultaneous spectrometers, which have different characteristics and are operated differently. The methods describe how to correct for spectral interferences, which are individual for the instruments. It is thus necessary to perform a spectral analysis during method development. The effectiveness of correction must then be checked by analysis of SRM's.

The instrumental methods of Loring and Rantala (1977) and Agemian and Chau (1975a) can be recommended for F-AAS. These methods use direct comparison of sample solutions with pure (mixed) standards made in the same acid matrix. Matrix modifiers are required for certain elements, and the two methods differ in this respect. Loring and Rantala's method has been validated by analysis of SRM's, whereas Agemian and Chau's has not. As suggested by Loring and Rantala, dilution factors (arising from digestion) of 100 - 2,000 can be applied as necessary.

The instrumental methods of Sturgeon et al. (1982) are recommended for determining Be, Cd, and Pb by GF-AAS. (The paper also gives methods for Cu, Ni, Co and Cr which probably do not need to be determined by GF-AAS.) The stabilized temperature plaform furnace (L'Vov platform) reduces matrix interference effects, eliminating the need for standard additions. The matrix modifiers and graphite furnace conditions given by Slavin et al. (1983) are also recommended. For many of the less volatile elements (including Ba, V, Cu and Ni) the L'Vov platform gives reduced sensitivity compared to a normal pyrolytically coated graphite tube. For these elements, the recommendations of Slavin et al.(1983) should be followed. The pyrolytic tube/matrix modifier/standard addition methods of Caravajal et al.(1983) and the methods of Sakata and Shimoda (1982) and Rantala and Loring (1980) for Cd also contain relevant information on GF techniques.

b) Biota

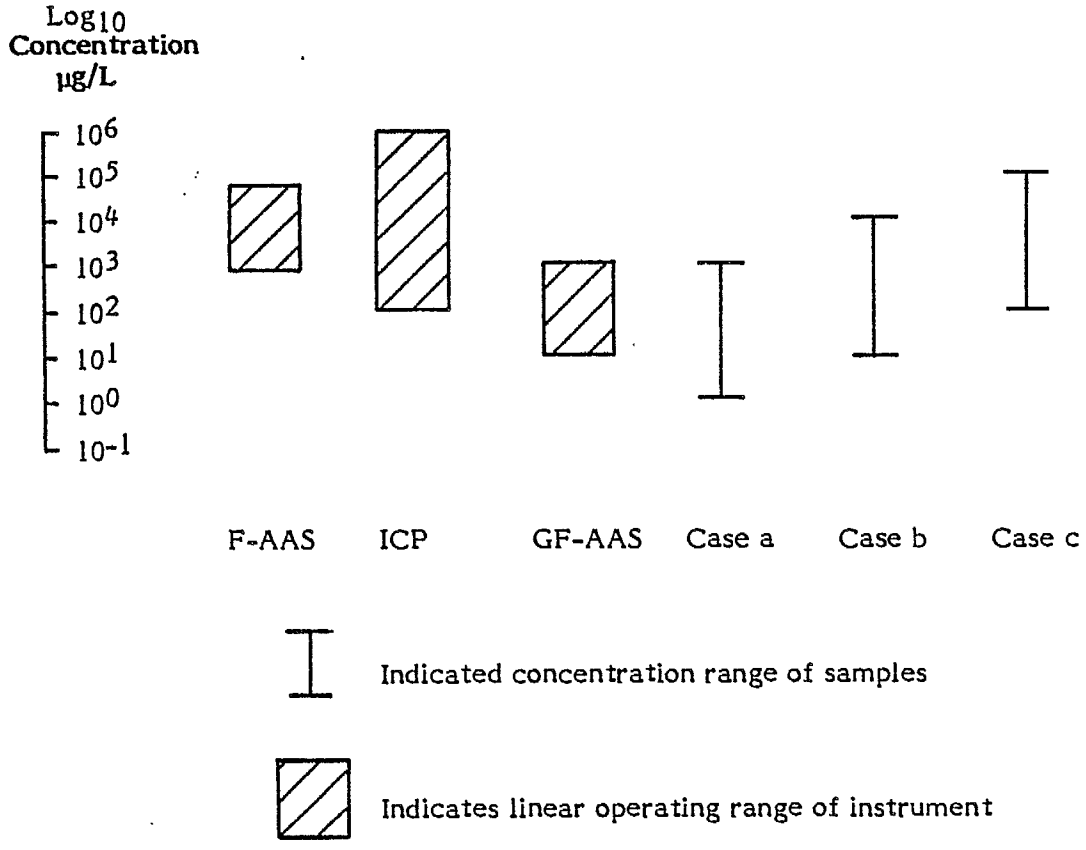
The general procedure for determining which instrumental techniques have sufficient, but not too much, sensitivity is described in section 6.3.1. In the analysis of tissues, it is assumed that digested tissue solutions can be analyzed either directly or after preconcentration. It is assumed that digestion will produce a 100 fold

dilution of the sample (final solution weight/original sample dry weight) and that preconcentration factors of 10 and 100 are possible. Thus, nine different combinations, 3 instruments and 3 solution concentrations, are evaluated. The solvent enhancement effect for aspiration of a combustible organic solvent into the flame of an AAS is not taken into account. The tissue concentration ranges the metals (µg/g, dry weight derived from Table III - 2.17 Volume I) are assumed to be:

As	.1 - 100	Hg	.002 - 10	Zn	2 - 2000
Cd	.01 - 10	Ni	.02 - 20		
Cr	.1 - 100	Pb	.03 - 30		
Cu	.1 - 100	V	.05 - 50		

These are generally less than the sediment concentrations, by factors of 10 -100.

Arsenic is taken to illustrate the procedure. The concentration range for As in tissues is 0.1 -100 µg/g. For digestion without preconcentration (case a), the final solution concentrations are 1-1000 µg/L. For digestion with 10x preconcentration (case b), they are 10-10,000 µg/L, and for digestion with 100x preconcentration (case c), they are 100-100,000 µg/L. The diagram below shows the instrumental operating ranges and final solution concentration ranges side by side.



It can be seen that As can be done by GF-AAS with a 10x preconcentration or by ICP with a 100x preconcentration.

The results for other metals are tabulated below.

	F-AAS		ICP		GF-AAS	
	Case b	Case c	Case b.	Case c	Case a	Case b
Too low	Hg	Hg	Hg	Hg	Hg	
Marginal	As,Cd,Ni, Pb,V	As,Pb	As,Cd, Ni,Pb	Pb	As,V, Ni,Pb	Hg
Within	Cu,Cr	Cd,Cr, Ni,V	Cr,Cu, V,Zn	As,Cd,Cr, Cu,Ni,V,Zn	Cu,Cr,Cd	As,Cd,Cr, Cu,Ni,Pb,V
Too high	Zn	Zn			Zn	Zn

From these results it can be concluded that if flame or ICP is to be used, a 100 fold preconcentration (case c) is necessary for most elements. If GF-AAS is to be used, then a 10x preconcentration (case b) is sufficient for most elements. A number of elements can be done by GF-AAS without preconcentration. The results for F-AAS may be biased somewhat due to neglect of the solvent enhancement effect, which can lower detection limits by a factor of 2 or more. These conclusions are generally consistent with the L.O.D.'s given for specific methods in Tables III-2.16 and 2.18 of Volume I, however.

The choice of flame, graphite furnace or ICP for the analysis of tissues is not restricted by sensitivity when preconcentration is allowed. Although it increases the cost of the analysis and adds to the difficulty, a preconcentration step is often unavoidable, since the graphite furnace is not sensitive enough to determine many of the elements directly. Preconcentration has the compensating advantage of reducing interference from Na, K, Ca and Mg, etc., making the analysis potentially more accurate. When a preconcentration step is required, it would be advantageous to use flame or ICP whenever possible. However, when the quantity of tissue that is available for analysis is very limited, the graphite furnace would be preferred since it requires considerably less (only 10-100 mg dry weight) tissue. Comparing ICP and F-AAS, ICP would be preferred when more than 3-5 elements are to be determined. This number of elements is a typical yield for a preconcentration method. Flame and ICP can be used together as an accuracy check on the instrumental determination.

RECOMMENDATIONS

The procedures given by Evans et al. (1980) and Evans et al. (1978) are recommended for F-AAS. These references contain useful information on the optimization of instrumental conditions as well as the accuracy, precision and detection limits obtainable. These procedures apply to the analysis of digested and preconcentrated tissue solutions. Some relevant information may also be contained in the papers by Loring and Rantala (1977) and Agemian and Chau (1975a), which describe the more difficult problem of the analysis of digested sediment solutions.

For the analysis of tissue preconcentrate by ICP, the procedures of Evans and Dellar (1982) are recommended. There is much useful information on instrumental conditions, calibration, interferences, and obtainable performance in this report. Jones et al. (1982) give little information on instrumental technqiues. For the analysis of tissue digestate by ICP, the information developed by McQuaker et al. (1979) is relevant. The information given by McLaren et al. (1981) and Floyd et al. (1980) on analysis of sediment digestate solutions may also be useful in developing an instrumental method for tissues. Spectral line interferences are more important in digestate than in preconcentrate solutions.

For the analysis of tissue digestate by GF-AAS, the stabilized temperature platform furnace (STPF) technique (Sturgeon et al. (1982), Fernandez et al. (1981) and Hinderberger et al. (1981)) is recommended. This technique applies especially to the more volatile elements such as Cd, Pb and As. For these metals, more accurate results will be obtained for less effort than atomization "off the wall" of a conventional graphite tube. Matrix modifiers are sometimes used in conjunction with the STPF; Slavin et al. (1983) gives graphite furnace conditions and matrix modifiers for a variety of elements. For the more refractory elements, e.g., Ba, Be, V, Cu and Ni, the STPF technique does not appear to offer any advantages. Borg et al. (1981) give instrumental settings for conventional graphite furnace determination of Cu, Pb and Cd in tissue digestate solutions. For determining Be in biological tissues, the preconcentration/GF-AAS method of Hurlbut (1978) is recommended.

6.3.3.2 Instrumental Methods for Arsenic in Sediments and Biota

From the standpoint of sensitivity, the hydride generation methods are all adequate for determining arsenic in sediments and tissues. Both AAS and ICP based

hydride methods are available. GF-AAS has sufficient sensitivity for determining arsenic in sediments without preconcentration, whereas a 10 fold preconcentration is necessary and sufficient for determining the lower levels of As found in tissues. ICP is almost sensitive enough to determine As in sediments, but not in tissues.

In terms of accuracy and precision, the hydride generation methods are better than the direct graphite furnace methods. After preconcentration by solvent extraction, GF-AAS will be much less subject to interferences. For sediments, direct GF-AAS is cheapest, especially when other (Group I) elements are determined on the same sample solutions. In commercial laboratories, HG-AAS or HG-ICP is about 2-3 times more expensive than direct GF-AAS, unless only arsenic is determined in which case the costs are about equal (Table III-2.14, Volume I). The cost of determining arsenic in tissues by GF after preconcentration would be similar to that for HG-AAS. HG-ICP has the advantage that several other hydride forming elements can be determined with arsenic for the same cost as determining arsenic alone by HG-AAS. From a brief survey of commercial laboratories in the Vancouver - Victoria area, it appears that instrumental set-ups for determining arsenic by hydride generation-AAS or ICP are common. The continuous hydride generation methods are superior in throughput and ease of operation, because they are automated. Though slightly less sensitive, the continuous methods should have a significant cost advantage whenever large numbers of samples are run routinely.

References to recommended instrumental methods are given in Table 6.1.

6.3.3.3 Instrumental Methods for Hg in Sediments and Biota

a) Total Mercury

CV-AAS techniques must be used for measuring mercury in sediments and biota. Flame AAS, GF-AAS and ICP have inadequate sensitivity. The automated or continuous CV-AAS methods have adequate detection limits for most sediment and tissue samples, but not for those having the lowest expected concentrations. The manual methods, which have detection limits that are about an order of magnitude lower, are adequate for the lowest concentrations. The automated methods will be preferred when sample concentrations are high enough and when large numbers of samples need to be analyzed, due to their higher throughput and slightly better precision. Automated instruments also require less attention to operate, but are more expensive and more difficult to set up.

Table 6.1

Instrumental Methods for Arsenic in Sediments and Biota

	HG-AAS	HG-ICP	GF-AAS
a)	Sediments		
	continuous: Agemian & Bedek (1980), Vijan et al. (1976) manual: Rubeska and Hlavinkova (1979)	continuous: deOlievera et al. (1983), Pahlavanpour et al. (1980), Goulden et al. (1981)	Caravajal et al. (1983) - direct GF-AAS method - matrix modifier, standard additions used Slavin et al. (1983) - STPF, Zeeman background corrector used
b)	Biota		
	continuous: Agemian and Thomson (1980), Arafat and Glooschenko (1981) manual: Evans et al. (1979), Kuldvere (1980)	continuous: Goulden et al. (1981) deOlievera et al. (1983)	Thiex et al. (1980) - solvent extraction/ GF/AAS method

The recommended instrumental methods are given in Table 6.2. The manual techniques exist in a variety of configurations (see Section III-2.3.3 - Volume I for descriptions), all of which have approximately the same limit of detection. The stationary method of Tong and Leow (1980) is somewhat slower than the other manual methods, but has the advantage of simplicity. The Perkin Elmer MHS-1 mercury/hydride system (Kuldvere and Andreassen, 1979) and the Varian model 65 vapour generation accessory (MacPherson et al, 1982) are easily obtained and seem to perform as well as the other manual methods.

b) Methylmercury

There are three instrumental techniques for determining methylmercury in fish tissue - gas chromatography with electron capture detection, CV-AAS and GF-AAS. Of the three, GC-EC is the most commonly used. The detection limits of the GC-EC and CV-AAS methods are similar (0.05 - 0.1 µg/g) while that of GF-AAS is higher. The GC-EC methods are superior to the others in selectivity, since the gas chromatograph is capable of resolving the organomercurial compounds while the other instruments cannot differentiate them. Assuming that the costs are approximately equal, GC-EC is preferred since it has better selectivity and equal or better sensitivity. CV-AAS would be preferred if GC-EC were not available, since it is more sensitive than GF-AAS.

Before the above mentioned instrumental techniques can be used to quantify methylmercury it is necessary to employ a method to separate it from the sample. Preconcentration methods for methylmercury in tissues are discussed in Section 6.4.4.2. References for the instrumental methods are given with the recommendations for preconcentration methods.

6.4 Preconcentration Methods

6.4.1 General Considerations

As mentioned previously, preconcentration procedures are almost always applied to sea water, often applied to tissues and seldom applied to sediments. Sea water has extremely low concentrations of trace metals relative to major and minor cations, i.e. Na, K, Ca and Mg. Tissue digests have a composition of major and minor cations that is similar to seawater, but the trace metals are relatively more

Table 6.2

Instrumental Methods for Mercury in Sediments and Biota

	Automated CV-AAS	Non-automated CV-AAS
a)	Sediments	
	Agemian and Chau (1975b) - manifold system - 20 samples/hour sampling rate	MacPherson et al. (1982) - equilibrium partition method - Varian model 65 vapour generation accessory Ure and Shand (1974) - equilibrium partition method
b)	Biota	
	Agemian and Chau (1975b) Agemian and Cheam (1978) - manifold system - 20 samples/hour sampling rate	Kuldvere and Andreasson (1979) - Perkin Elmer MHS-1 mercury/ hydride system Chapman and Dale (1978, 1982) - equilibrium partition method - transfer of Hg vapour by displacement with water Tong and Leow (1980) - stationary CV-AAS method

concentrated in tissues. Sediment digests have a rather different composition of major and minor elements (e.g. Fe, Al and Si are much higher), and analyte concentrations are higher in sediments than in tissues. The high concentrations of Fe and Al can pose problems in applying preconcentration methods to sediments.

Preconcentration methods are sometimes needed:

1) **to bring the analytes into the operating range of the instrument and**

2) **to remove interferences arising from the matrix of major and minor elements.**

The use of a preconcentration step increases the cost of the analysis and should not be used unless it can be justified. How sample concentration and instrumental detection limits can be used to decide which instruments are appropriate for an analysis has been described in Section 6.3.1. Which instruments are appropriate also depends on whether a preconcentration method is available. The decisions to use particular instrumental and preconcentration methods thus interact.

Interferences arising from the major and minor elements are very common. Although they can sometimes be mediated by special instrumental techniques, such as matrix modifiers and special temperature programs on the graphite furnace, using a preconcentration method is usually the most effective way to deal with the problem, because the interfering elements are almost entirely removed without affecting the concentration of analytes. Interferences are common but are very hard to anticipate since they depend on the particular combination of analyte and interfering elements and are caused by a variety of physical and chemical mechanisms. In general, the potential for interference diminishes in the order: GF-AAS > F-AAS > ICP. In the case of ICP, interferences are mainly of the spectral type, for which exact corrections can be made.

Preconcentration methods are generally indifferent to the (total) digestion method used since the interfering cations are affected in the same way by the different digestions and the anions introduced by the acids usually do not interfere with the preconcentration methods. Similarly, since the interfering alkali and alkaline earth metals are almost completely removed from the solution containing the analytes, instrumental methods are generally not sensitive to the preconcentration method used. Thus preconcentration methods can generally be

considered to be interchangeable. There is no essential difference between the methods that are used for GF-AAS and ICP, for example.

Concentration factors resulting from preconcentration methods are variable; they may be as low as 5 or as high as 500. The concentration factor can be adjusted to suit the needs of the particular analysis situation, e.g., it may be adjusted to enable a particular limit of detection to be achieved. An upper limit for the concentration factor exists for every preconcentration technique. As this limit is approached the separation becomes less quantitative. The limits for concentration factors are not usually given in methods reports and some experience is required to estimate them. The limiting concentration factors can be obtained by experiment, and thus concentration factors can be regarded as a variable under the control of the experienced analyst.

Most preconcentration methods are multielement methods. Up to nine metals can be co-extracted from sea water using dithiocarbamate chelating agents. There is a substantial increase in efficiency when a single preconcentration suffices to do a number of elements, since the preconcentration step can consume a large portion of the analysis time.

6.4.2 Preconcentration Methods for Interstitial Waters

6.4.2.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

As previously mentioned, preconcentration methods are usually necessary for measuring trace metals in sea water and interstitial water. Preconcentration serves two important purposes: removal of the interfering alkali and alkaline earth metals and raising the concentration of the analytes to be within the operating range of the instrument. **The principal techniques used for sea water are: 1) solvent extraction, 2) chelating ion exchange separation and, 3) coprecipitation.**

Of these solvent extraction is certainly the most popular. Solvent extraction offers the following advantages: broad scope, speed, simplicity, high efficiency, high concentration factors and low and reproducible blanks. Most of the lowest detection limit methods combine GF-AAS with solvent extraction preconcentration. A two-fold solvent extraction in which the metals are first extracted into an organic solvent and later back-extracted into nitric acid is preferred, because it produces higher extraction efficiency, greater concentration and the final extract is stable against

concentration change with time. In addition, dilute nitric acid is more compatible with GF-AAS and ICP, than are organic solvents.

New chelating ion exchange methods have recently been developed which employ a variety of different chelating functional groups. The chelating ion exchange techniques potentially offer the same advantages as the two-fold solvent extraction techniques, but being newer they are less widely used. Also, some of the new chelating ion exchange resins are not commercially available and must be prepared in the laboratory. The older Chelex 100 technique is still used despite certain drawbacks, and Chelex based methods have recently been improved. Coprecipitation is less important than the other two techniques. It is apparently less developed and fewer multi-element methods are available. Some important single element methods employ coprecipitation, however.

In order to adopt a seawater method for interstitial water analysis, it will usually be necessary to scale it down to use smaller sample volumes. This will usually involve a proportional reduction of all volumes. It may also involve using smaller flasks, pipettes, separatory funnels, extraction columns, etc. Centrifugation may be used instead of filtration. Some useful information can be gleaned from reports (cited in Volume 1) of seawater methods being adapted for interstitial water analysis. Such adaptations may require some trial and error, but in most cases they should be fairly easy to accomplish.

RECOMMENDATIONS

The multielement preconcentration methods have been summarized in Table 6.3. **The two-fold solvent extraction procedures given in the table are recommended for the following reasons.** They are based on a well proven and firmly established technique. The range of metals that can be extracted is as great as chelating ion exchange and greater than Co-APDC coprecipitation. Very high (500 fold) preconcentration factors and very low blanks are possible. The overall performance of the two-fold solvent extraction methods is probably as good or better than any other available method.

The chelating ion exchange method of Sturgeon et al. (1981) offers comparable performance to the two-fold solvent extraction methods, but the chelating ion exchange material (8-hydroxyquinoline chemically bonded to silica gel) must be prepared in the laboratory. Until this material is widely available, the

Table 6.3

Recommended Multielement Preconcentration Methods for Seawater

a) Solvent Extraction Methods

Reference	Metals	Chelating Agents/Extraction Solvents
Danielsson et al. (1978), Magnussen and Westerlund (1981), Danielsson et al. (1982)	Cd, Co, Cu, Fe, Ni, Pb, Zn	APDC + DDDC/Freon TF;Nitric Acid
Bruland et al. (1979)	Cd, Cu, Ni, Zn	APDC + DDDC/Chloroform; Nitric Acid
Sturgeon et al. (1980b)	Cd, Cr, Cu, Ca, Fe, Mn, Ni, Pb, Zn	APDC + Oxine/MIBK;Nitric Acid
Sugimae (1980)	Cd, Cu, Fe, Mn, Ni, Pb, V, Zn	NaDDC/Chloroform; Nitric Acid
Miyazake et al. (1982)	Cd, Cu, Fe, Mn, Mo, Ni, Pb, V, Zn	APDC/Di-isobutyl ketone
McLeod et al. (1981)	Cd, Cu, Fe, Mo, Ni, V, Zn	APDC + DDC/Chloroform; Nitric Acid

b) Chelating Ion Exchange Methods

Reference	Metals	Chelating Ion Exchange Material
Sturgeon et al. (1981)	Cd, Co, Cu, Fe; Mn, Ni, Pb, Zn	Silica immobilized 8-hydroxyquinoline
Sturgeon et al. (1980a), Bruland et al. (1979), Kingston et al. (1978)	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn	Chelex 100

c) Coprecipitation Methods

Reference	Metals	Precipitate
Boyle and Edmond (1975), Boyle and Edmond (1977), Klinkhammer (1980b), Klinkhammer (1982)	Cu, Ni, Cd, Fe	Cobalt APDC

method will probably not be widely adopted for routine use. The Chelex 100 methods are slower and are not able to extract colloidal or organically bound species of Cu and Ni from sea water.

A number of single element methods exist for Cr despite the fact that it can also be determined by multielement methods. The importance of the single element methods is that two of them can determine both species, Cr (III) and Cr (VI). Cranston and Murray's (1978) method relies on coprecipitation with $Fe(OH)_3$ while that of de Jong and Brinkmann (1978) utilizes solvent extraction with Aliquot-336. The single element methods for Cr do not seem to offer any significant advantages over the multielement methods of Sturgeon et al. (1980a). The preconcentration/GF-AAS methods of Rollemburg and Curtius (1982) and Burba et al. (1983) are indispensible for Ba and Be, respectively, since neither of these two elements can be satisfactorily determined by any other methods found in Volume 1. Single element methods exist for Mn and for V, but these do not seem to offer any significant advantages over the multielement methods which include Mn and V (Table 6.3).

6.4.3 Preconcentration Methods for Sediments

Preconcentration methods for sediments are uncommon and are usually not needed since most metals can be satisfactorily determined directly in the solutions resulting from digestion. Two preconcentration methods for sediments were found, however. Sturgeon et al.'s (1982) method uses solvent extraction to separate Cd, Pb, Cu, Ni and Co from the matrix. A pre-extraction is required to remove Fe and Al which would otherwise interfere, and this results in partial loss of Cd. The method of Sanzolone et al. (1979) also uses solvent extraction to separate Co, Ni and Cu. Interference from Fe and Al ions is avoided by masking them with fluoride.

These methods may be useful for bringing the low concentrations of Cd and Pb into the operating range for ICP or F-AAS and for reducing matrix interferences, but they add substantially to the analysis time. Cd and Pb can be satisfactorily determined by GF-AAS, and it is not necessary to remove matrix elements from dissolved sediments by solvent extraction to obtain satisfactory results.

6.4.4 Preconcentration Methods for Biota

6.4.4.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

Preconcentrations are often applied to bring digested tissue samples into the analytical range of the instrument. As indicated in Section 6.3.4.1, a preconcentration step is needed for determining most metals in tissues by F-AAS or ICP. Preconcentration is also needed for determining certain metals in tissues by GF-AAS. Preconcentration separates the analytes from potentially interfering alkali and alkaline earth elements. Since the matrix of potentially interfering ions in tissues is very similar to that for sea water, the methods used in these two instances are also very similar. In general, preconcentration methods for sea water could be transferred with little difficulty to tissues and vice-versa. The preconcentration methods for tissues are summarized in Table 6.4. As with sea water, the main techniques are solvent extraction and chelating ion exchange.

RECOMMENDATIONS

The methods of Evans et al. (1978) and Agemian et al. (1980) which aspirate the organic solvent directly into the flame, are recommended for F-AAS. Okuno et al.'s (1978) method back-extracts the metals into nitric acid and is recommended for analysis by GF-AAS. None of these methods are appropriate for ICP, which would require complete removal of the organic solvent by evaporation and redissolution in nitric acid. Concentration factors of 10 are typical for these methods but factors of 100 could probably be attained judging from what is typical for seawater methods. It seems likely that solvent extraction methods developed for sea water could be readily applied to tissue digest solutions, thereby enabling higher preconcentration factors and more metals to be extracted.

The Chelex 100 method of Jones et al. (1982) is suitable for ICP or GF-AAS. When HF is used in the digestion, fluoride interferes with retention by forming soluble complexes with several elements. Jones et al.'s method has the advantage of covering a broad spectrum of metals and the disadvantage of being slower than most solvent extraction methods.

Table 6.4

Multielement Preconcentration Methods for Tissues

REFERENCE	METALS	CHELATING AGENTS/ EXTRACTION SOLVENTS
a) Solvent Extraction Methods		
Evans et al. (1978)	Cd, Pb, Ni	APDC + DDDC/4-methyl-pentan-2-one
Agemian et al. (1980)	Cd, Pb, Ni	APDC/MIBK
Okuno et al. (1978)	Cd, Pb, Cr	APDC/MIBK; Nitric Acid

REFERENCE	METALS	CHELATING ION EXCHANGE MATERIAL
b) Chelating Ion Exchange Methods		
Jones et al. (1982)	Al, Cd, Co, Cu, Mo	Chelex 100
Kingston et al. (1978)	Ni, Pb, V, Zn	

6.4.4.2 Methylmercury

There are two techniques for separating methylmercury from tissues for instrumental quantification by GC-EC, CV-AAS or GF-AAS. These are solvent extraction and steam distillation. Solvent extraction methods have been used for 20 years and are well established. There are two variations, that of Krenkel and Burrows (1975), which is standard, and the recent method of Hight and Capar (1983), which incorporates changes to make it simpler and faster. The Hight and Capar (1983) method was proven in a collaborative study and has been adopted by the Association of Official Analytical Chemists (A.O.A.C.). The steam distillation method of Collett et al. (1980) offers some advantages over the solvent extraction methods. It is faster and easier to perform; however, it may or may not result in as pure a product. The two methods are described in some detail in Section 2.4.4 of Volume I.

RECOMMENDATIONS

Comparing the complete methods, the solvent extraction/GC-EC methods of Krenkel and Burrows (1975) and of Hight and Capar (1983) seem to offer the most advantages. The steam distillation/CV-AAS method of Collett et al. (1980) seems to be equally sensitive but less selective. The steam distillation technique could perhaps be successfully combined with GC-EC, but this has not yet been proven.

6.5 Digestion Methods

6.5.1 General Considerations

The type of digestion method depends on the type of sample to be digested. The aluminosilicate minerals in sediments require HF to dissolve or else high temperature fusion with an alkaline flux. Organic matter can be mineralized with strong oxidizing acids such as HNO_3 and $HClO_4$. Alternatively, tissues can be ashed in a high or a low temperature furnace. The destruction of metal-organic complexes or organometallic compounds in interstitial waters can be accomplished under milder oxidizing conditions.

Within each broad category of sample type, there are variations in sample composition which necessitate variations in digestion procedure. For example, plant tissues sometimes contain silica, which requires HF to dissolve, while animal tissues usually do not. Sediments exhibit varying concentrations of organic matter and sometimes contain refractory minerals. Interstitial waters may or may not contain natural organic ligands which interfere with solvent extraction or chelating ion exchange preconcentration.

A decision that must be made in the analysis of sediments is whether to measure the total metal concentrations, the concentrations which are removed with strong concentrated acids or to measure the concentrations which are leached by some weaker and perhaps more selective form of chemical attack. The available choices and the considerations involved in making a choice are discussed in Section 6.5.3.1. Methods which measure particular metal species are sometime available; that option usually exists for As and Hg. Whether particular metal species are to be measured strongly influences the choice of digestion method.

The choice of a digestion method is influenced by the choice of instrumental technique, since the instruments are somewhat sensitive to the acids used, the viscosity of the solution, concentration of dissolved solids, etc. Whether a preconcentration step is needed to boost sensitivity and eliminate interferences also affects the choice of digestion method. The chemical properties of the metals to be determined may influence the choice of digestion method. The volatility of As and Hg compounds places restrictions on the conditions that can be employed in digestion procedures for these metals.

After sample type, type of attack (for sediments) or particular species, and instrumental technique have been considered in that order, final discrimination can be made on the basis of accuracy (including contamination, losses and completeness of digestion), cost (e.g., speed, ease, special equipment, etc.) and safety. It is sometimes difficult to evaluate methods according to these criteria because the information available is incomplete. Tables have been assembled to facilitate an objective choice when a wide variety of methods is available.

6.5.2 Interstitial Waters

6.5.2.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

Bruland et al. (1979) found that Chelex 100 would not extract unreactive forms of dissolved trace metals from sea water and Danielsson et al. (1982) reported

that their two-fold solvent extraction method manifested this interference in low salinity estuarine water samples. Although evidence of this problem occurring for interstitial waters is lacking so far, it seems likely to occur. **Therefore, it is recommended that either of the two successful digestive pretreatment procedures employed by Danielsson et al. (1982) be applied to interstitial water samples when total metal concentrations are to be determined.** These procedures consist of heating the sample in the presence of acid and H_2O_2. **When a digestive pretreatment is not used prior to solvent extraction, the determination should be called "extractable metal concentration".**

6.5.2.2 Arsenic

Since arsenic species are usually determined rather than the total concentration of arsenic, a digestion is not appropriate.

6.5.2.3 Mercury

Without oxidative pretreatment to liberate organo-mercury compounds or other unreactive forms of Hg, the CV-AAS methods of Olafsson (1983) and Bloom and Crecelius (1983) will measure the "easily reducible" fraction of Hg in the sample. For determining the total Hg concentration, Olafsson recommends oxidation with $KMnO_4 + K_2S_2O_8$ at 80C for 2 hrs. Bloom and Crecelius (1983) found that the bromine monochloride (cold) oxidation method of Szakacss et al. (1980) gave better results than the $KMnO_4 + K_2S_2O_8$ hot oxidation and the acid aging methods. The bromine monochloride method has a lower blank than the permanganate-persulphate method and is more effective than acid aging. A fourth alternative, the ultraviolet photooxidation method was successfully employed by Fitzgerald (1975).

6.5.3 Sediments

6.5.3.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

Three kinds of digestions can be distinguished on the basis of whether it is desired to liberate all or part of the metals contained in the sediments:

a) **Total attack methods** determine the "total metal concentration", although incomplete dissolution is sometimes a problem for sediments containing refractory minerals or a large quantity of organic matter. Total attack procedures use HF in combination with other strong concentrated acids or else high temperature fusion with an alkaline flux and acid dissolution of the melt. Since determination of total concentration is intended, the accuracy of the determination can be assessed. This is important when comparison with a legal standard is necessary. The main disadvantage of most total attack methods is the unpopularity of HF.

b) **Strong attack methods** employ strong concentrated acids other than HF. Strong attack is usually seen as an alternative to total attack that is operationally less difficult but yields concentration values that are roughly comparable (for some metals in some sediments). One would normally expect almost complete dissolution of clay minerals, hydrous oxides and organic matter. The advantages of strong attack methods are: the use of HF is avoided; and they yield lower concentrations of salts, acids and interfering substances (such as Fe, Al and Si), resulting in a solution that is easier to quantify instrumentally. The disadvantage is that there is no way to assess accuracy. However, precision and reproducibility are usually good. Some workers feel that the results of strong attack are of limited value in assessing metal contamination as the attack removes some but not all of the metals incorporated in crystal lattices as well as metals derived from anthropogenic sources. Other workers feel that it is unnecessary to obtain complete digestion of all sedimentary components, including aluminosilicate minerals, since pollution effects usually occur on the surfaces of particles and in allochthonous particles.

c) **Moderate and weak attack methods** are usually aimed at determining the more readily available, biogeochemically important fraction of metals in sediments. They employ weaker and possibly more selective chemical attack agents, such as 0.05M EDTA, 2% sodium dithionite or 1M ammonium acetate. Three main purposes for using these kinds of digestion have evolved: to survey anthropogenic metal concentrations in sediments, to measure the 'biologically available' metal concentration, and for studying elemental

partitioning. These methods can give some insight into the nature of the partitioning of elements among different sedimentary components (such as organic matter, carbonates and aluminosilicate minerals). They can be criticized on the grounds that they yield a value which is operationally defined and difficult to interpret. Perhaps a more important criticism is that they are less rugged than total or strong attack methods, i.e. more sensitive to variations in procedure.

RECOMMENDATIONS

a) Total Attack Methods

Acid attack methods are generally preferred to fusion attack for trace metals, because acid attack produces a solution which contains lower concentrations of acids and salts and which subsequently has lower blanks and fewer interferences. Instrumental technique is the most important factor affecting the choice of total acid attack method. **If F-AAS is to be used, it is sufficient to employ a conventional Teflon bomb or beaker digestion with HF plus aqua regia or HF plus nitric-perchloric acid followed by neutralization of the excess HF with boric acid. The methods of Loring and Rantala (1977) and Agemian and Chau (1975b) are of this type.** Dilution factors of 100-1000 are typical for this kind of digestion. If ICP or GF-AAS will be used however, it is preferable to substitute a careful evaporation step for treatment with boric acid to rid the solution of excess HF. Subsequent evaporations can be used to reduce the excess quantity of other acids used (such as perchloric) enabling final redissolution in 1M HCl. Thus the final solution is almost ideal for instrumental quantification. The methods of McLaren et al. (1981), Sturgeon et al. (1982) and Brzezinska et al. (1984) make use of careful evaporation steps in place of boric acid. A dilution factor as low as 50 can be achieved in this way. Whenever combinations of instrumental techniques are used to quantify different metals in the same solutions, the latter type of digestion method is recommended. Although the evaporation steps are more time consuming and difficult to perform, they are necessary to obtain the best results from GF-AAS and ICP.

Contamination of sediments during digestion is not likely unless for the very low level elements such as Pb, Cd, As and Hg. The use of Teflon bombs or Teflon beakers in a clean room or clean bench lessens the risk. Also the use of high purity acids and reagents in minimum quantities helps. Completion digestion can probably be effected with less acid in a Teflon bomb than in a Teflon beaker.

The older literature (e.g., Agemian and Chau, 1975c) alludes to possible loss of Cr as CrO_2Cl_2, but more recent experience with digestion methods which include evaporation steps seems to indicate that evaporation steps, carefully applied, do not cause any losses, even for arsenic. Thus, volatility does not seem to be a problem for total acid attack methods, and a Teflon bomb is not better than a Teflon beaker in this respect. Mercury, however, can be lost if the mixture of acids is not sufficiently oxidizing, and Si will be lost (as SiF_4) from an open vessel digestion with HF. Losses do not seem to be very limiting for the $LiBO_2$ fusion method of Owens and Gladney (1976); complete recovery of Ba, Be, Co, Cr, Cu, Mn, Mo, Pb, V and Zn was obtained from NBS fly ash.

Incomplete dissolution can sometimes be a problem when using total acid attack methods on sediments containing resistant minerals such as barite and chromite, or an especially high content of organic matter. The usual acid attack methods sometimes give values that are too low for Ba. **For determining barium in sediments, especially those containing barite ($BaSO)_4$) , a fusion method (Owens and Gladney, 1976; Mahan and Leyden, 1983; Caravajal et al., 1983) is recommended.** Chromite may also resist complete dissolution by total acid attack methods (note the differing obtained results for Cr on BCSS-1 and MESS-1 by Sturgeon et al., 1982 and McLaren et al., 1981). Whenever incomplete dissolution of refractory minerals is a concern, a fusion method should be used, since fusion attack is more powerful. When sediments are very high in organic matter content, the HNO_3 + $HClO_4$ + HF method of Hsu and Locke (1983) may be preferred, since the acid mixture used by this method is more oxidizing than that of Loring and Rantala or Agemian and Chau and is thus claimed to be more effective. From the standpoint of completeness of digestion, a Teflon bomb is probably superior to a Teflon beaker, - the bomb can be used at higher temperature and pressure. Grinding the sediments to a fine powder also increases the rate and extent of dissolution.

b) **Strong Attack Methods**

The alternative to total attack is usually strong attack with some combination of HCl, HNO_3, $HClO_4$ or H_2SO_4. The instrumental techniques are generally insensitive to which strong attack method is used. The choice of instrumental technique for strong attack follows the same logic as that for total attack; sensitivity is the main consideration. Strong attack methods are expected to liberate 10% - 100% of the total metal concentrations.

The aqua regia method of Agemian and Chau (1976) or the HNO_3 + HCl (9 + 1) method of Sinex et al. (1980) will suffice for most purposes. The more elaborate and time consuming procedure of Caravajal et al. (1983) seems unnecessary. For samples that contain unusually high amounts of organic matter, a more oxidizing combination of acids, such as $HClO_4$ + HNO_3 (1 + 1) (Agemian and Chau, 1976) or $HNO_3 + H_2O_2$ (10 + 3) (Krishnamurty, 1976) may be preferred. Sulphuric acid should perhaps be avoided, because sulphates may interfere with GF-AAS determination and precipitation of $PbSO_4$ is possible. **Grinding of sediments is not recommended when strong attack methods are used,** because grinding is unnecessary and introduces errors which tend to decrease precision and reproducibility.

c) Moderate and Weak Attack Methods

The choice of a specific method depends mainly on the objectives of the study, whether it is to survey anthropogenic metals, to measure the amount that is biologically available or to give information about strength of metal binding and association with particular sediment components. The results of moderate and weak attack methods are sensitive to sampling and sample storage procedures; the advice given by Thomson et al. (1980) should be followed. Because partial and selective extraction procedures sometimes are not very rugged, precise control over extraction time and temperature may be necessary. Long extraction times (8 hours) can also be used to minimize experimental error (Maher, 1984).

For pollution surveys, the 25% acetic acid leach of Loring (1981) and the 0.5M HCl leach of Chester and Voutsinou (1981) have proven to be effective. Agemian and Chau (1977) and Malo (1977) preferred 0.3-0.5M HCl for measuring authigenically formed metals in sediments. Luoma and Bryan (1981) recommended 1N HCl to provide a measure of nondetrital metal concentration. A selective extraction method which measures only anthropogenic and not natural forms of anthigenic metals is not available. Methods for measuring bioavailability is beyond the scope of the methods review; a single example is Luoma and Bryan (1981). Geochemical partitioning methods also were not given detailed coverage. Sources found in the Review are: van Valin and Morse (1982), Tessier et al. (1979), Engler et al. (1977), Luoma and Bryan (1981) and Maher (1984).

6.5.3.2 Digestion Methods for Arsenic in Sediments

a) **Total Attack Methods**

Three techniques are represented:

1) NaOH (or KOH) fusion (Goulden et al., 1981; de Oliveira et al., 1983)

2) $LiBO_2$ fusion (Mahan and Leyden (1983); Caravajal et al., 1983)

3) Acid digestion with HF (Agemian and Bedek, 1980; de Oliveira et al., 1983).

Accurate results were obtained for As in SRMs using all three techniques.

RECOMMENDATIONS

The acid digestion technique is preferred for arsenic for the same reasons that acid digestion is preferred to fusion attack for total metal determinations for other metals (convenience and lower salt/acid matrix). The use of an HF digestion for arsenic has the additional advantage that other elements can be determined on the same final solutions. The methods of Agemian and Bedek (1980) and de Oliveira et al. (1983) are compatible with both GF-AAS and HG-AAS (or HG-ICP) techniques. The use of a Teflon bomb is recommended to prevent loss of arsenic (III) halides (de Oliveira et al., 1983); otherwise oxidizing agents such as permanganate and persulphate are needed (Agemian and Bedek, 1980).

b) **Strong Attack Methods**

Numerous strong attack methods for determining arsenic in sediments are available.

1) $HCl + HNO_3$ (Caravajal et al., 1983)

2) $HNO_3 + HClO_4 + H_2SO_4$ (de Olievera et al., 1983; McLaren et al., 1981)

3) $HNO_3 + HClO_4$ (Vijan et al., 1976)

4) Aqua regia (Rubeska and Hlavinkova, 1979)

5) HCl (in sealed test-tubes) (Pahlavanpour et al., 1980).

Strong attack methods usually give a close approximation to the total arsenic concentration. Since accuracy cannot be assessed for strong attack methods, efficiency or convenience may facilitate a choice. **On that basis, the method of Pahlavanpour et al. (1980) excels, since it has the highest throughput and uses the least objectionable acid.**

c) Moderate and Weak Attack Methods

Maher's (1981) method is the only method in this category. It can be used to determine inorganic and methylated species of arsenic in sediments. The digestion procedure consists of an initial leach with 1M HCl followed by extraction with 0.1M NaOH + 11M NaCl. Most of the inorganic arsenic is extracted with the acid leach and methylated forms are found only in the alkaline solution.

6.5.3.3 Digestion Methods for Mercury in Sediments

The digestion is a critical step in the determination of total mercury in sediments. Strong attack methods are usually employed, and total attack is very uncommon. It is usually assumed that very little mercury is contained in aluminosilicate minerals, and it has been shown by Agemian and Chau (1975a) that HF is not necessary for determining total mercury in sediments. Essentially all of the mercury can be liberated using strong attack methods. However, some errors can still arise in strong attack digestion for mercury in sediments. For example, organomercury compounds may be volatilized by the high temperature required to oxidize the organic matter. Incomplete dissolution of cinnabar (HgS) is another source of error that has been suggested.

Elaborate strong attack digestion procedures have been developed for determining mercury in sediments to obviate these sources of error. The method of Agemian and Chau (1976) for example digests the sample with H_2SO_4 + HNO_3, and a trace of HCl is added to dissolve cinnabar. Then $KMnO_4$ and $K_2S_2O_8$ are added to ensure complete oxidation of the sample and of all forms of mercury. Hydroxylamine hydrochloride is then added to reduce the excess permanganate and persulphate,

immediately before $SnCl_2$ is added to reduce mercuric ions to elemental mercury. Because this procedure uses so many reagents, it is more difficult to obtain a low blank.

More recently there has been a trend toward less complicated procedures which eliminate the auxiliary oxidization and the pre-reduction step. The method of Randlesome and Aston (1980), for example, merely uses HNO_3 + HCl. It is not known whether Randlesome and Aston's (1980) method can always be relied upon to give accurate results. The HNO_3 + HCl method of MacPherson et al. (1982) however, has been well validated using BCSS-1 and MESS-1. An auxiliary oxidant ($K_2Cr_2O_7$) is used, but the method is less complicated and less time consuming than Agemian and Chau's (1976) method.

RECOMMENDATIONS

The elaborate digestion procedure of Agemian and Chau (1976) can be used with the automated CV-AAS methods while MacPherson et al.'s (1982) simpler digestion procedure is appropriate for the manual CV-AAS methods.

6.5.4 Biota

6.5.4.1 Al, Ba, Be, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn

There are basically, three techniques for digesting tissues:

1) wet oxidation,
2) high temperature ashing and
3) low temperature ashing.

Wet oxidation is by far the most commonly used technique. It is usually accomplished with a mixture of oxidizing acids such as HNO_3 + $HClO_4$ + H_2SO_4, carried out at relatively low temperatures, < 140°C, and is probably the safest with respect to volatilization of analytes. Since wet oxidation uses more acids than high and low temperature ashing, contamination from reagents is more problematic. Variations in the concentration of residual acid may be an interferent. High concentrations of residual acid may reduce the response of F-AAS and ICP. Incomplete dissolution is

sometimes a problem, e.g. with high fat content samples. Safety can be a concern when employing wet oxidation methods.

High temperature ashing in a muffle furnace is second in popularity. Dissolution of the ash can be accomplished with less acid and therefore blanks are potentially lower. The high temperatures used (500-550°C) may volatilize some elements and fixation to solid components of the system can occur. Low temperature ashing is accomplished with atomic oxygen at low pressure in a special oxygen plasma furnace. Like high temperature ashing, low temperature ashing uses smaller quantities of reagents, and the lower temperature (100-140°C) is the main advantage of the technique. Its lower popularity can probably be attributed to the initial expense of the equipment, newness of the technique and its slowness relative to the muffle furnace technique.

Acid digestion of tissues is in some respects more difficult than sediments. Tissues are more variable in the problems they present, hence acid dissolution methods are more variable. Open or closed vessel digestions, single or successive additions of acids, with or without evaporation to dryness, constant temperature or temperature programs, various combinations of acids, etc. are some sources of variation in wet oxidation methods. Metal concentrations are generally lower in tissues than in sediments, by as much as one or two orders of magnitude, so contamination and reagent blanks are more problematic. Digesting tissues is more hazardous than digesting sediments. The evolution of CO_2 and H_2O makes digestion of tissues in closed containers difficult and there is more risk of an explosion.

In order to facilitate choosing a method from the variety that are available, the methods have been analyzed and classified according to certain criteria in Table 6.5. The criteria require some explanation. Sample type is an important factor because different kinds of samples to some extent require different kinds of digestions. Plant tissues sometimes require HF whereas animal tissues do not, and specialized methods have been developed for high fat content animal tissues. The method of digestion should be matched as closely as possible to the type of sample for which it is appropriate. The particular combination of acids used is given in the table. The original references must be consulted for the detailed procedures.

Which elements were measured, the instrument that was used and whether preconcentration was employed are given in the table. Digestion methods need not be considered as suitable only for the elements measured and the instrumental techniques used. The effectiveness of a digestion method can be judged on the basis of the extent to which its accuracy has been verified and, on the basis of the

TABLE 6.5

Digestion Methods for Plant and Animal Tissues

Reference	Jones et al. (1982)	Brzezinska et al. (1984)	Feldman (1974)	Agemian et al. (1980)	Borg et al. (1981)	Cary and Rutzke (1982)
Sample Type	Plant or animal	plant or animal	plant or animal	animal	animal	plant
Acids Used	$HClO_4 + HNO_3$ or $HClO_4 + HNO_3 + H_2SO_4$ (post-digestion of insoluble silicates with HF)	Plant: $HCl + HNO_3 + HClO_4$ + HF (method a) or $HNO_3 + HClO_4$ + HF (method b) Animal: $HNO_3 + HClO_4$ (methods a & b)	$HClO_4 + HNO_3 + H_2SO_4$	$HNO_3 + H_2SO_4 + H_2O_2$	$HNO_3 + H_2O_2$	$HClO_4 + HNO_3 - H_2SO_4$ (post-digestion of insoluble silicates with HF)
Metals	alkali and alkaline earth, Fe, Mn, Al, P, Cd, Cu, Cr Co, Mo, Ni, V, Zn, As, Se, Sb	Ca, Mg, P, Fe, Mn, Al, Zn, Cu, Mo, Ni, Pb, Co, Cr, Cd, As, Se, Sb, Ag	Hg	Cu, Cr, Cd, Pb, Zn	Cu, Cd, Pb, Zn	Cr
Instrument/ Preconcentration	ICP/Chelex 100	ICP	CV-AAS	F-AAS/solvent extraction	GF-AAS	GF-AAS/$Fe(OH)_3$ coprecipitation
Effectiveness	- Accuracy verified for 7 SRMs - Results varied for binary and ternary acid mixtures and different metals - Most plant and some animal tissues required HF to obtain complete dissolution	- Accuracy verified for 5 SRMs - Complete dissolution assured with HF - Method a more sensitive but more open to contamination	- Accuracy verified for several SRM's - Immune to loss by volatization - low risk of contamination - Versatile procedure depends on sample type	- Accuracy verified for 3SRMs - Complete dissolution of high fat content fish tissue observed	- Accuracy verified by analysis of NBS bovine liver and by comparison with neutron activation analysis - Not suitable for volatile metals metals such as Hg and Se	- Accuracy vrified for 5 SRM's - Complete dissolution observed
Efficiency	- Kjeldahl flasks and heating rack - 2 step temperature program for ternary acid mixture	- Both methods use Teflon bombs in a pressure cooker - Method a: successive additions and evaporations; 3-4 hours - Method b: single addition, no evaporation; 2 hours	- Volumetric flasks fitted with air condensers on a hot plate; - Condenser assures retention of Hg vapour while allowing diltillation of decomposition vapours - successive additions, temperature programming; takes 1-1/2 - 2-1/2 hours, can process 5-20 samples simultaneously	- Digestion tube in an aluminum heating block - Successive additions and evaporations - Includes a charring step	- Digestion tubes in an aluminum heating block - Successive additions and evaporations; 24 hours - Clean laboratory or filtered air hood recommended	- Digestion tubes - Time not given

information that is given on contamination, loss or completeness of digestion. Efficiency can be judged by how rapid, difficult or otherwise costly the method appears to be. This evaluation is also constrained by the amount of information contained in the methods reports. Because the effectiveness of a digestion procedure for a particular combination of metals determined, sample type and instrumental technique cannot be predicted, definitive recommendations are not given. It seems likely that most combinations will work; the obvious exceptions are few. Therefore, the recommendations are intended to be general, in order to provide a sufficiently broad scope to satisfy most needs.

RECOMMENDATIONS

For digesting animal tissues, a variety of different kinds of methods can be recommended. Methods which use combinations of HNO_3 + $HClO_4$ + H_2SO_4 are the most common. Feldman et al. (1974) and Jones et al. (1982) used these acids in Kjeldahl type flasks on a hot plate or heating rack. Five to 20 samples can be processed simultaneously in less than 3 hours. The air jacketed condenser used by Feldman (1974) is not necessary for most metals. Feldman's (1974) method has 3 variations and is therefore the most versatile. Brzezinska et al. (1984) used HNO_3 + $HClO_4$ in Teflon bombs heated in a pressure cooker. Depending on the concentration levels of the analytes, two variations, one which includes evaporation steps and one which does not, can be applied. If it is desired to avoid the use of $HClO_4$, methods employing HNO_3 + H_2SO_4 + H_2O_2 can be used. Agemian et al. (1980) and Borg et al. (1981) used combinations of these acids with digestion tubes in an aluminum heating block. The longer time required by Borg et al.'s (1981) method is not necessarily a disadvantage, since most of the time is unattended. Agemian et al.'s (1980) method includes a charring step which may be objected to by some.

For digesting plant tissues, the $HClO_4$ + HNO_3 + H_2SO_4 + HF methods of Jones et al. (1982) and Cary and Rutzke (1983) can be recommended. The HNO_3 + H_2SO_4 + H_2O_2 method of Arafat and Glooschenko (1981) has the drawback that complete dissolution was not obtained, probably because HF was not used.

6.5.4.2 Digestion Methods for Arsenic in Tissues

a) For Determining Total Arsenic Concentrations

There are many methods (11) available for determining total arsenic concentrations in biological tissues. Most of these gave accurate results for arsenic in SRMs. One was not properly validated and another gave results that were too low.

It is controversial whether wet or dry ashing techniques should be used for determining As in samples of marine origin. Both techniques have been applied successfully. Some evidence has been advanced that if a wet digestion method is used which does not have sufficient oxidizing power to keep arsenic in the pentavalent oxidation state, loss of As might result.

RECOMMENDATIONS

For animal tissues, the nitric-perchloric-sulphuric acid methods of Thiex (1980) and Agemian and Thomson (1980) are recommended. This is probably the most popular combination of acids for digesting animal tissues, and it is sufficiently oxidizing to prevent volatilization of As (III) compounds. The effectiveness of the 2 methods has been well validated. The method of Agemian and Thomson (1980) which utilizes the aluminum heating block technique, is probably the more efficient. The methods are suitable for use with HG-AAS (Agemian and Thomson, 1980) and solvent extraction/GF-AAS (Thiex, 1980). The dry ashing method of Evans et al. (1979) is a viable alternative to the two wet ashing methods. $Mg(NO_3)_2$ is used as an ashing aid and to ensure complete recovery of As. It has been well validated and is suitable for HG-AAS.

The recommended methods for plant tissues are the $H_2SO_4 + HNO_3 + H_2O_2$ methods of Kuldvere (1980) and Arafat and Glooschenko (1981). The latter obtained accurate results for As in three SRMs and is suitable for HG-AAS. The procedure, which uses Erlenmeyer flasks and a hot plate, would be more efficient if modified to use digestion tubes in an aluminum heating block. Kuldvere's (1980) method, which was applied to seaweed, uses the aluminum heating block technique and is also suitable for HG-AAS.

b) For Determining Arsenic Species

There are three methods available for determining arsenic species (As III, As V, monomethylarsonic acid and dimethylarsinic acid) in biological tissues. These employ selective extractions rather than acid digestion to separate arsenic compounds from the biological matrix. Andreae (1978) extracted homogenized tissues with 2M HCl. Maher (1981) found that extraction with 0.1M NaOH yielded better recoveries than 1M NaOH, 7.5M HCl or water and did not degrade the methylated species to inorganic arsenic. The methylated species accounted for a significant fraction of total As in seaweed. Aggett and Kadwani (1983) extracted homogenized plant tissues with distilled water, methanol and ethanol. Inorganic arsenic was extracted with water, while significant amounts of methylated species were extracted with methanol or ethanol.

RECOMMENDATIONS

It is clear from these studies that the above mentioned extractants can be used to separate arsenic species from homogenized tissues and that the extractions are somewhat selective for the different species. As in the use of selective or partial extraction methods for sediments, the task of recommending partial extraction methods that are appropriate for particular purposes falls outside the scope of this guide.

6.5.4.3 Digestion Methods for Mercury in Tissues

The choice of digestion method for determining total mercury in tissues appears to be crucial for obtaining accurate results. A variety of different techniques are available, and they fall into two categories - those which effect complete oxidation of organic matter and those which do not. Another distinction which can be made is whether oxidation is accomplished under acidic or basic conditions. All of the methods except one were applied to fish tissues.

The available methods are listed below:

a) Complete Oxidation Methods

Feldman (1974)	$HClO_4 + HNO_3 + H_2SO_4$
Agemian and Chau (1975b, 1976)	$H_2SO_4 + HNO_3$; $KMnO_4 + K_2S_2O_8$
Agemian and Chau (1978)	$H_2SO_4 + H_2O_2$; $KMnO_4 + K_2S_2O_8$
Velghe et al. (1978a)	$H_2SO_4 + KMnO_4$
Chapman and Dale (1982)	1.5M KOH; $KMnO_4$; H_2SO_4

b) Incomplete Oxidation Methods

Tong and Leow (1980)	30% NaOH + 0.6% NaCl
Velghe et al. (1978b)	H_2SO_4

The complete oxidation methods employ a pre-reduction step (using hydroxylamine or oxalic acid) and are therefore generally more time consuming. The elaborate procedure of Feldman (1974) appears to be safest with respect to loss of mercury by volatization during digestion. Only the methods of Feldman (1974), Chapman and Dale (1974) and Tong and Leow (1980) have been properly validated using SRM's. On this basis it appears that a rigorous oxidative digestion is not necessary to obtain accurate results. The method of Tong and Leow (1980) appears to be as effective, and it is significantly faster.

RECOMMENDATIONS

The method of Feldman (1974) is preferred, since it seems to be the most effective (in terms of contamination, loss, completeness of digestion) yet is probably not more time consuming than the other complete oxidation method that has been successfully validated (Chapman and Dale, 1982). The method of Tong and Leow (1980) is less time consuming, but it may objected to by some because it is not rigorous. Nevertheless it has been well validated.

6.6 Cost of Analysis

Approximate costs for the main types of analyses are given in Table 6.6. These are based on 1984 prices obtained from several commercial laboratories in the

Vancouver-Victoria, B.C. area. For AAS, the cost per sample depends on the number of elements determined. For ICP however, a flat rate is charged for 20 or 30 elements determined. Thus it is much cheaper to do a large number (20-30) of elements by ICP (as reflected by this table), the cross-over being in the neighborhood of 3-5 elements. The ranges reflect the variability among laboratories, which is large. The methods used by the different laboratories varies somewhat which accounts for some of the variability.

Table 6.6
Per Sample Cost of Analysis

	Group I Metals			As	Hg
	F-AAS	GF-AAS	ICP-AES	HG-AAS	CV-AAS
Sediments	$60-100[1]	$90-115[1]	$15-50[1,4]	$10-25	$10-25
Biota	$60-100[2]	$90-115[2]	$15-50[2,4]	$10-25	$10-25
Interstitial Water	-	$50- 75[3]	-	-	-

1 Total acid digestion and direct determination of the 10 Group I metals.

2 Acid digestion and direct determination of the 10 Group I metals. For a method which includes a preconcentration step, add $10-20.

3 Solvent extraction preconcentration and GF-AAS determination of 5 Group I metals.

4 ICP determination includes 10 Group I metals plus 10-20 other elements.

7.0 METHODS FOR THE ANALYSIS OF ORGANICS

This section outlines selection criteria for choosing appropriate methods for the determination of selected organic compounds in marine sediments and benthic organisms. The following types of analyses are included:

1. Total Organic Carbon (TOC)
2. Chemical Oxygen Demand (COD)
3. Petroleum Hydrocarbons
4. Oil and Grease
5. Phenols
6. Chlorophenols
7. Chlorinated Hydrocarbons (Pesticides and PCBs)

The recommendations given are based on the review of methods in Volume 1.

7.1 Total Organic Carbon (TOC)

TOC analyses are two step procedures involving the conversion of organic carbon to CO_2 and measurement of either the quantity of oxidant consumed for the conversion or the CO_2 evolved. Organic carbon can be converted to CO_2 by either wet oxidation (with potassium dichromate/sulfuric acid or potassium persulfate) or dry combustion (heating at 550 - 1600°C in the presence of O_2). The evolved CO_2 can be determined gravimetrically, volumetrically, by infrared absorbance or by thermal conductivity measurements. Analytical methods for TOC determinations are reviewed in Section III-3.2, Volume 1.

Two methods are recommended for TOC analysis of sediments: a low cost method (wet oxidation-titration) and a high accuracy method (acid digestion - dry combustion). The detection limit of the first method is 1 $mg \cdot g^{-1}$ while that of the second is approximately 250 $ug.g^{-1}$. These methods are outlined in Table 7.1.1 and 7.1.2 respectively and discussed below.

Table 7.1.1

Determination of TOC in Sediments by Wet Oxidation/Titration

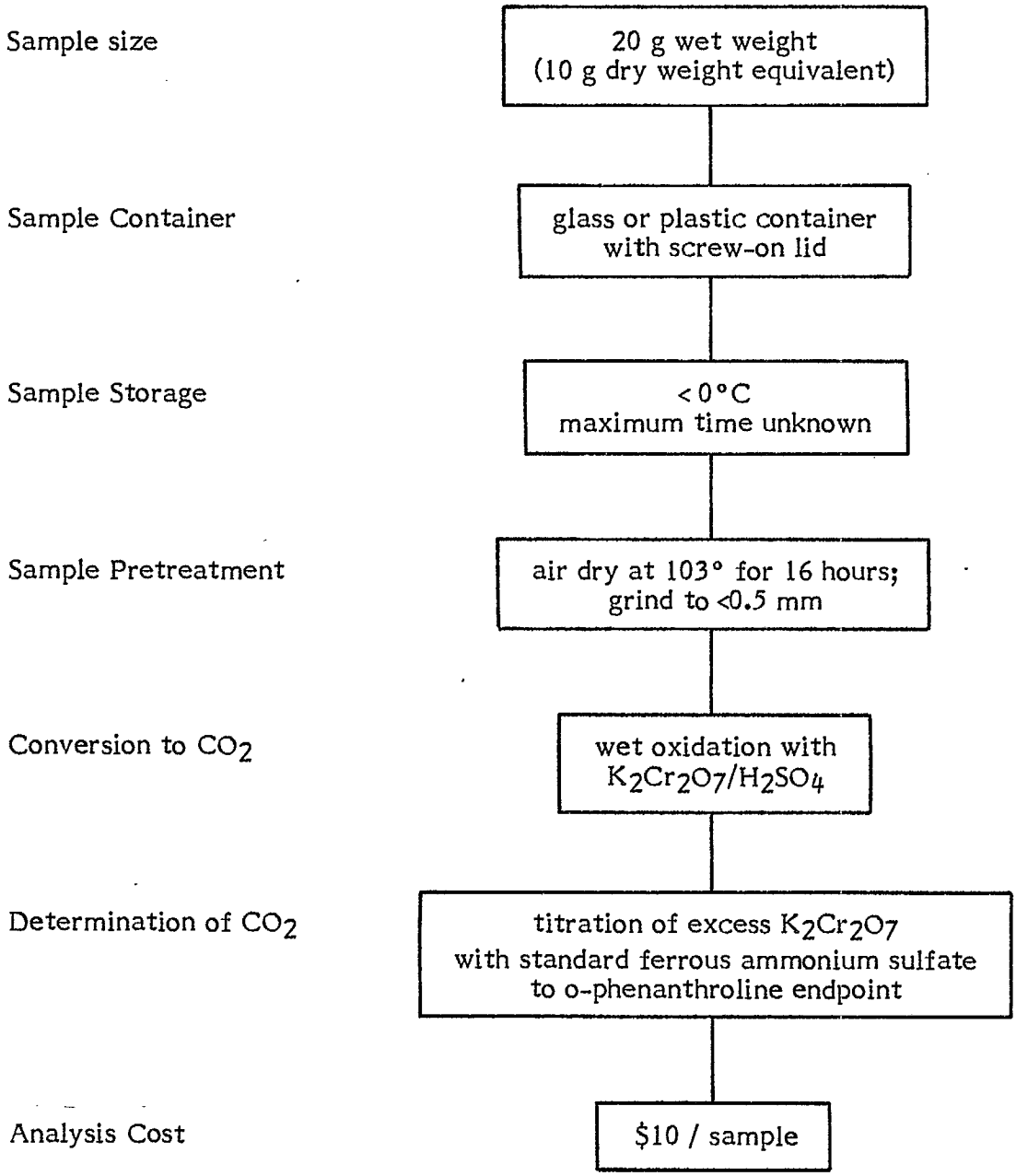
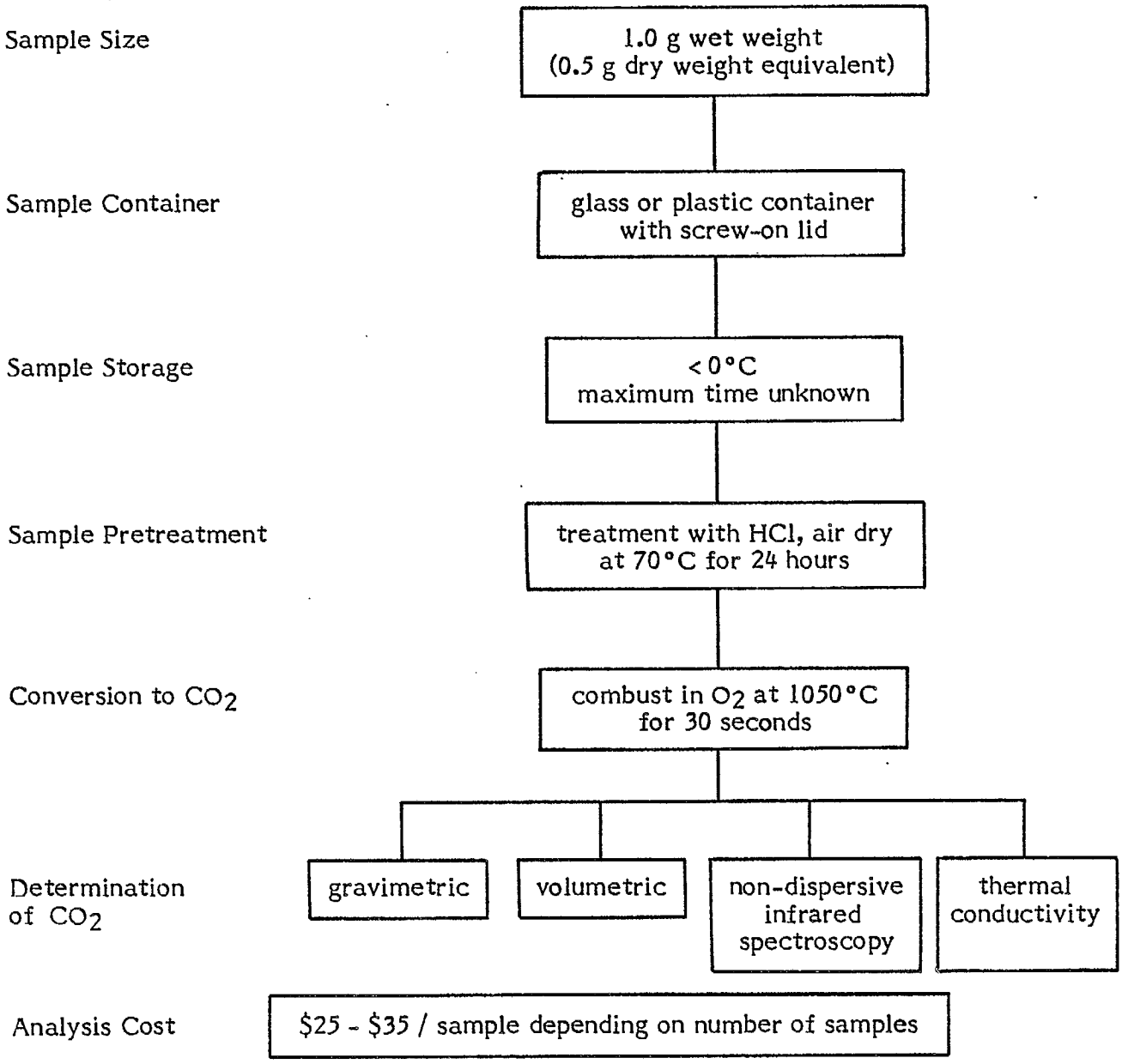


Table 7.1.2

Determination of TOC in Sediments by
Acid Digestion/Dry Combustion



7.1.1 Sample Requirements

Up to 20 grams wet weight of sediment is required for TOC determination by the wet oxidation-titration procedure (EPS, 1979). The exact sample size required is determined by its organic carbon content (10 to 25 mg of organic carbon per sample aliquot analyzed). Approximately one gram wet weight of sediment is required for TOC analysis by the acid digestion-dry combustion method.

Glass or plastic containers can be used for sample storage (EPS, 1979; Plumb, 1981). Samples should be stored moist at 4°C to minimize loss of organic compounds through volatilization. Maximum times for sample storage are unknown but should be minimized to avoid loss due to chemical or biological oxidation (Plumb, 1981). One month is suggested as a reasonable storage time.

7.1.2 Wet Oxidation - Titration Procedure

The Walkley-Black wet oxidation-titration method is recommended for use as an inexpensive determination of the TOC content of sediments (EPS, 1979). The advantages of this procedure include its low analysis cost, low capital cost (only standard laboratory equipment is required) and widespread historical use. However, results by this method may be an inaccurate indicator of absolute organic carbon content of the sediment and should be considered only as a procedurally-defined quantity. EPS recommends designation of results as "Organic Carbon, chromic acid oxidation values". Inaccuracies arise from

i) the incomplete oxidation of organic carbon (between 70 and 90% complete, depending on the refractory carbon content of the sediment);

ii) standardization difficulties (the organic carbon may be at a different overall oxidation state than the organic carbon used to standardize the titration); and

iii) the non-specificity of the oxidation towards carbon (oxidizable interferences include iron (II), manganese (II), sulfide, and to some extent, chloride ions).

The standardization and non-specificity errors can be minimized by directly measuring the CO_2 evolved by the wet oxidation. This however requires special equipment or adaptation of commercial instruments adding significantly to the capital cost, the analysis cost and the inconvenience. Such a modification is not warranted for this analysis as it eliminates the advantages of the wet oxidation technique while ignoring the errors due to incomplete oxidation of the organic carbon in the sample.

The EPS procedure calls for drying of the sediment sample before analysis. The drying technique is not critical (freeze-drying, air-drying or oven-drying at temperatures between 60 and 180°C are acceptable). The drying step may result in some loss of volatile organic compounds from the sample and caution should be exercised if such components are expected to be major constituents of the organic material in the sediment.[1]

The organic carbon in the sample is oxidized by treatment with a potassium dichromate/sulfuric acid solution. This reagent produces higher oxidation efficiencies than potassium persulfate. The excess oxidant is measured by titration with standard ferrous ammonium sulfate to an o-phenanthroline endpoint. The organic carbon content of the sample is calculated from the number of equivalents of potassium dichromate used.

7.1.3 Acid Digestion - Dry Combustion Procedure

A dry combustion procedure is recommended for accurate determination of the organic carbon content of sediment samples. Such a method is more specific for carbon as it measures the quantity of evolved CO_2 directly and complete oxidation of organic carbon can be achieved by selection of appropriate combustion conditions.

To maximize the accuracy of the TOC value, organic carbon should be determined directly rather than by subtraction of inorganic carbon values from total carbon values. Two methods of differentiating organic carbon from inorganic carbon are used:

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[1] Total organic carbon is defined as carbon in non-volatile organic compounds (Plumb, 1981).

i) prior removal of inorganic carbon (i.e., carbonate and bicarbonate) from the sample by acidification or

ii) a differential combustion method in which organic compounds are selectively oxidized under mild conditions.

The combustion of inorganic and organic carbon at overlapping temperatures may cause significant error in a differential combustion method. Therefore removal of inorganic carbon by acidification with a non-oxidizing acid such as H_3PO_4 or HCl (Plumb, 1981) is the recommended method. The acidification method provides a good partition between organic and inorganic carbon but has some potential for experimental error (sample can be lost by frothing or acid-soluble organic carbon (SOC) may be lost by leaching through permeable crucibles or if a filtration is carried out). The EPA method (Plumb, 1981) details the steps required to avoid such errors (slow addition of acid, drying rather than filtration).

Dry combustion is carried out in a stream of O_2 using an induction or a resistance furnace (Plumb, 1981). Combustion temperatures ranging from 550 - 1600°C have been reported in the literature. Gibbs (1977) recommends combustion at 1050°C or greater for at least thirty seconds.

Dry-oxidation TOC analyses are generally carried out using commercially available instrumentation that couples a combustion system to a CO_2 measuring system. Such systems can be volumetric, gravimetric, thermal conductivity or infrared absorbance. All provide accurate measurement of evolved CO_2 and selection should be made on the basis of availability and cost of the various instruments. Volumetric, gravimetric and thermal conductivity methods require prior separation of interfering gases (such as H_2O, SO_2 and Cl_2) while infrared measurements are specific to CO_2.

7.2 Chemical Oxygen Demand

Chemical Oxygen Demand (COD) is a procedurally-defined quantity used as an indicator of organic material in sediments. The sediment sample is digested with a strong oxidizing agent at elevated temperatures and low pH and the amount of oxidant consumed is measured and related to an equivalent amount of O_2. **A standard method for COD determinations on sediments has been published by the EPA (Plumb, 1981) and is recommended for use.**

7.2.1 Sample Handling and Storage

Approximately 2 grams wet weight of sediment is required for COD analysis. Samples are stored wet in precleaned (detergent wash, distilled water rinse) glass or plastic containers, minimizing air contact and storage headspace to prevent air oxidation. Recommended storage temperature is 4°C with a time limit of one week (Plumb, 1981). No pretreatment or drying of the sediment is required.

7.2.2 Method of Analysis

As COD is a procedurally-defined quantity, the standard method (EPA: Plumb, 1981) should be followed exactly. The sediment is refluxed with a known amount of potassium dichromate ($K_2Cr_2O_7$) solution. The excess $K_2Cr_2O_7$ is measured by titration with standard ferrous ammonium sulfate to a ferroin endpoint. The $K_2Cr_2O_7$ consumed is converted to an equivalent quantity of O_2 and called COD.

7.3 Petroleum Hydrocarbons

Many types of analyses for petroleum hydrocarbons can be performed on environmental samples, ranging from a qualitative analysis in which the samples are simply screened for the presence of petroleum compounds to accurate quantification or detailed compositional description of the petroleum present. As shown in Table 7.3.1, several techniques can be used to obtain each of these various types of information on sediment and biota extract solutions. The choice of analytical method for a particular analysis is therefore based on several factors which are predetermined by the user of the analytical results and are dependent on the goals of the analysis program being carried out. These factors include:

1) accuracy
2) sensitivity (or detection limit)
3) the compositional information required and
4) the cost.

The analytical techniques used in petroleum analysis are rated in terms of these factors in Table 7.3.1 and discussed in detail in Section III-3.4 of Volume I.

Table 7.3.1

Methods for Petroleum Hydrocarbon Determination Rated According to Sensitivity, Accuracy, Level of Compositional Information and Cost

METHOD	QUANTITATION			COMPOSITIONAL INFORMATION	COST*
		Accuracy			
	Relative Sensitivity	Without Extract Cleanup	With Extract Cleanup		
gravimetry	low	low	high	none	$ 10
infrared spectroscopy	low	low	low	aliphatic content, functional group analysis	$ 15
microgravimetry	moderate	low	high	none	$ 15
ultraviolet spectroscopy	moderate	low	low	aromatic content	$ 15
UV-fluorescence spectroscopy	high	moderate	moderate	aromatic content	$ 15
gas chromatography/ flame ionization detector	high	low	high	boiling point distribution OEP, pristane/phytane ratio, n-alkane/isoprenoid ratio, major and minor component identification, alkylated PAH distribution	$40-100
gas chromatography/ mass spectrometric detection	high	moderate	high		$80-200

* Based upon analysis for petroleum hydrocarbons of one fraction of an extract dissolved in a solvent compatible with the method, but does not include the cost of sample extraction, clean-up and fractionation. Instrumental calibration costs are averaged over a suite of 5 samples. Where composition information is also generated, the cost includes data collection for a range of options, from minimal compositional information (just total petroleum hydrocarbons) to a full suite of parameters typically of interest.

A series of specific sensitivity/accuracy goals for the quantification of petroleum hydrocarbons in sediment and biota is presented in Table 7.3.2, along with the instrumental techniques which can be used to achieve each of these goals. Similarily, the techniques which can be used to provide compositional information on the petroleum in extracts are shown in Table 7.3.3 The recommended procedures for each sensitivity/accuracy and compositional analysis goal are discussed in the following sections.

7.3.1 Sample Requirements

Approximately 50 g wet weight of sediment (25 g dry weight equivalent) or 50 g wet weight of tissue (5 - 15 g dry weight equivalent) is required for petroleum hydrocarbon analysis. Although each individual method specifies a particular sample weight requirement (ranging from 1 g to 200 g), this is mostly a matter of convenience. It has been suggested (Hoffman et al., 1979) that subsampling errors are a primary contributor to poor correlations between petroleum hydrocarbon determinations by various methods. Due to the inhomogeneity of environmental samples, it is recommended that reasonably large samples (of approximately the same size) be taken for analysis and that a subsampling of the (liquid) extract be carried out if required for subsequent steps of the analysis. This will decrease sample size contributions to any variability in analytical results between the methods.

7.3.2 Storage Containers and Storage Conditions

Several types of containers are suitable for storing sediment samples for hydrocarbon analysis. Glass, aluminum, Teflon and stainless steel containers can be used but plastic containers and utensils and rubberized or waxed lids must be avoided (Clark and Brown, 1977). **Glass jars with teflon-lined lids are recommended.** However, aluminum foil-lined lids may be sufficient for analyses that do not require a high degree of accuracy. Although aluminum foil is rapidly deteriorated by the sediment (exposing the plastic lid and glue to the sample) it is less expensive and readily available. Glass containers and all glassware used in the analysis must be precleaned with detergent, rinsed with acetone and heated at 350°C for sixteen hours (Clark and Brown, 1977). Teflon or aluminum foil used for lining lids must also be washed in a non-polar solvent such as hexane.

Table 7.3.2

Methods for the Determination of Total Petroleum Hydrocarbon Content in Sediment and Biota Extracts

Goal	Techniques	Potential Application
A. Screening	gravimetry, IR, UV, UV-fluorescence	- identification of contaminated samples, i.e. determination of a real extent of damage
B. Low Sensitivity/ Low Accuracy	IR, UV, UV-fluorescence, microgravimetry	- estimation of level of contamination
C. Low Sensitivity/ Moderate Accuracy	microgravimetry, UV, UV-fluorescence, GC/FID, GC/MS	- monitoring trends in contamination levels as a function of time and/or location
D. High Sensitivity/ Moderate Accuracy	GC/FID, GC/MS	- monitoring trends in low level contamination as a function of time and/or location
E. High Sensitivity/ High Accuracy	GC/FID, GC/MS	- baseline levels
F. Low Molecular Weight Hydrocarbons	GC	- measuring levels of contamination

Table 7.3.3

Methods for Obtaining Petroleum Hydrocarbon Compositional Information on Sediment and Biota Extracts

Goal	Techniques	Potential Application
Aromatic content	UV, UV-fluorescence, GC/MS GC/FID on aromatic fraction, gravimetry on aromatic fraction	- determination of source of input e.g. petroleum pollution vs. incomplete combustion of fossil fuels
Aliphatic content	IR, GC/MS, GC/FID on aliphatic fraction, gravimetry on aliphatic fraction	
Boiling point distribution or distillation curve	GC/FID	- identification of source as refined product - determination of degree of weathering of contaminant i.e. new vs. chronic pollution
OEP	GC/FID, GC/MS	- distinction of biogenic from anthropogenic inputs
Pristane/phytane ratio	high resolution GC/FID, GC/MS	- distinction of biogenic from anthropogenic inputs
n-alkane/isoprenoid ratio	high resolution GC/FID, GC/MS	- determination of degree of weathering of contaminant i.e. new vs. chronic pollution
major component analysis	GC/FID GC/MS	- determination of source of input - determination of degree of weathering of contaminant - distinction of biogenic from anthropogenic inputs
minor component analysis	GC/MS	- determination of source of input - determination of degree of weathering of component
parent and alkylated aromatic homolog distribution	GC/MS	- determination of source of input e.g. petroleum pollution vs incomplete combustion of fossil fuels

Biota samples should be wrapped tightly with hexane-rinsed aluminum foil and stored in a container (not plastic) with a tight fitting lid (Clark and Brown, 1977). Some people have recommended rinsing of the biota with a solvent to remove surface petroleum, but this should not be necessary for benthic biota and may even result in some loss of petroleum hydrocarbons.

Both sediment and biota samples should be maintained tightly capped and in a frozen state until analysis (Clark and Brown, 1977). The effect of storage time on sample integrity is unknown but it has been suggested as a contributing factor to variability of results in interlaboratory comparisons. **It is therefore recommended that sample storage time before analysis be minimized and be less than six months for maximum accuracy and precision of results.**

No pretreatment of the sample is required. Samples are thawed immediately prior to analysis and analyzed wet or dry. There is some concern over loss of petroleum compounds during air-drying, oven-drying or freeze-drying procedures so drying is avoided when maximum accuracy is required. Drying of the samples is discussed below for each analysis procedure recommended.

7.3.3 Semi-Quantitative Screening for Petroleum Hydrocarbons

A rapid and inexpensive semi-quantitative analysis can be performed on sediment and biota samples to detect the presence of petroleum hydrocarbons. The recommended analysis procedure for sediment samples is outlined in Table 7.3.4 and the procedure for biota in Table 7.3.5 . These methods are suitable for detection of moderate and high levels of petroleum contamination (greater than approximately 50 $\mu g \cdot g^{-1}$ in sediment and tissue). Other methods must be used if lower detection limits are required (see Sections 7.3.2 to 7.3.4).

7.3.3.1 Detection of Petroleum Hydrocarbons

Gravimetry, infrared, ultraviolet (UV) spectroscopy or UV-fluorescence can be used to detect petroleum compounds in sediment and biota extracts. Although these techniques are comparable in cost and availability, gravimetry and infrared spectroscopy are unable to distinguish petroleum from biogenic and diagenetic material in the extract. This distinction can be made if the extract solution is subjected to cleanup procedures prior to the petroleum determination. The infrared method is also susceptible to interference from water, imposes limitations on the

Table 7.3.4

Screening Sediments for Petroleum Hydrocarbons

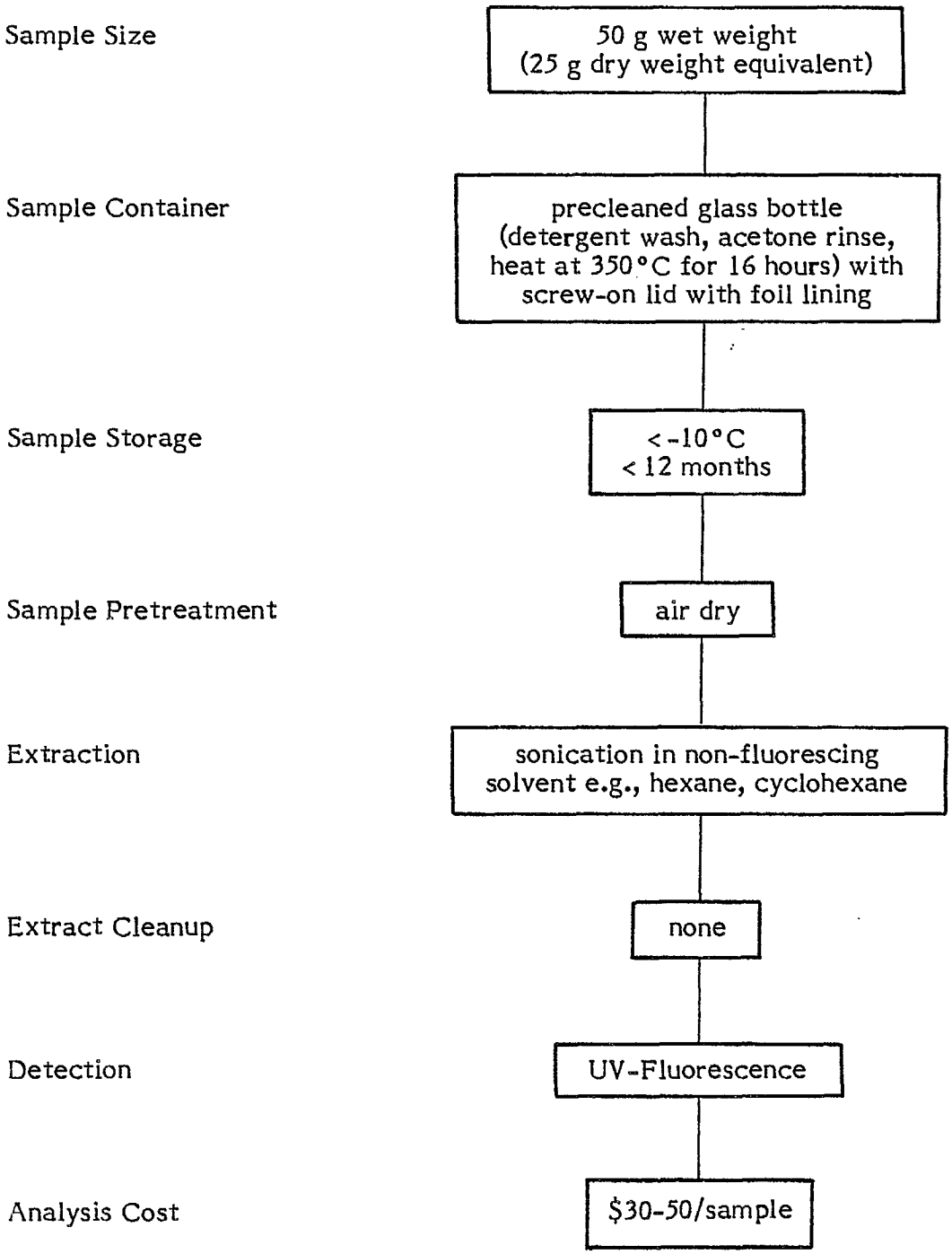
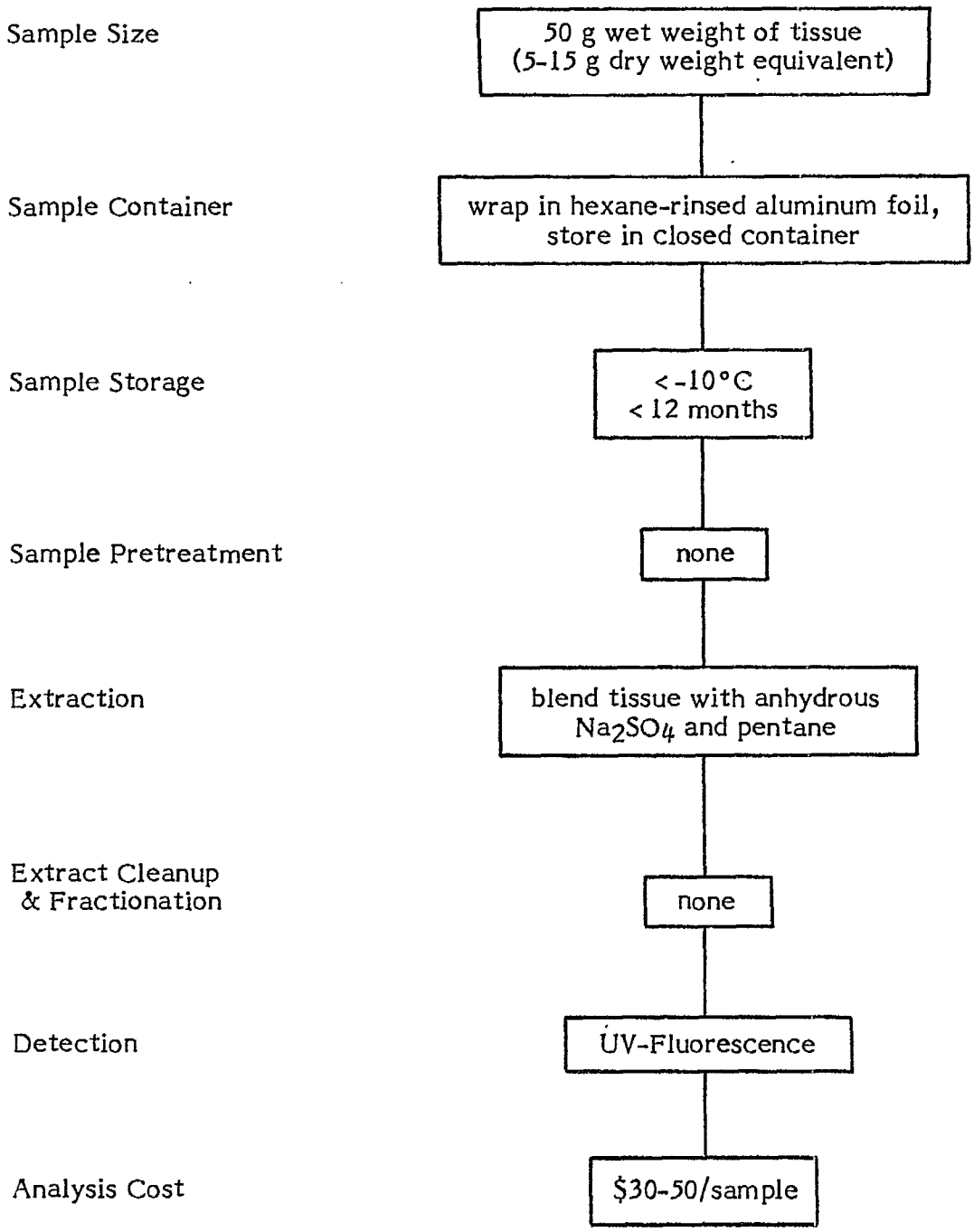


Table 7.3.5

Screening Biota for Petroleum Hydrocarbons



choice of extraction solvent and is strongly biased in favour of saturated hydrocarbon compounds. UV spectroscopy and UV-fluorescence are more specific for petroleum as they are biased in favour of aromatic compounds. Both UV and UV-fluorescence measurements can be obtained on the whole (uncleaned, unfractionated) extract, thus reducing the overall analysis cost. However, fluorescence is the preferable technique as it is more specific and can detect lower levels of petroleum contamination.

The application of UV-fluorescence to the determination of petroleum hydrocarbons in sediment and biota extracts is described by Hoffman et al. (1979) and Fong (1976) respectively. Synchronous scanning UV-fluorescence measurements should be made if the instrumentation is available as it has the ability to differentiate background pigment contributions to the observed fluorescence. Otherwise fixed wavelength UV-fluorescence measurements can be obtained. These instruments are less expensive and more commonly available than the synchronous scanning instruments, but there is more possibility of interference from co-extracted material when fixed wavelength measurements are used.

7.3.3.2 Sediment Extraction

Pre-drying of the sediment is recommended for the semi-quantitative screening analysis. Loss of hydrocarbons during the drying of the sediment is likely to be insignificant to the outcome of the analysis if it is carefully carried out. Air-drying is the recommended procedure (Overton and Laseter, 1980). The analysis of pre-dried sediment permits the use of a non-polar solvent for extraction, minimizing the amount of co-extracted polar lipid material and facilitating adjustment of extract solution volumes prior to the detection step.

Any extraction technique listed in Table III-3.12 Volume 1 can be used for a screening analysis. Some of these methods, while very efficient at extracting petroleum compounds from the sample matrix, are time consuming and contribute significantly to the total analysis time and cost. They may also result in large quantities of co-extracted material which interfere in the subsequent detection of petroleum hydrocarbons. Selection of an extraction method is therefore based on speed and cost of analysis rather than on efficiency of extraction. The method of Tan (1979) satisfies these requirements. Pre-dried sediment is extracted by sonication in a non-polar solvent such as cyclohexane. The choice of extraction solvent is dependent on the instrumental technique used for detection of petroleum hydrocarbons.

Solvents for UV fluorescence analysis are limited to those which do not fluoresce or quench fluorescence and saturated hydrocarbons (usually hexane, iso-octane or cyclohexane) are recommended. If other solvents are used for extraction, such as freon or dichloromethane, they must be exchanged to a suitable hydrocarbon solvent prior to UV-fluorescence analysis.

When UV-fluorescence is used for detection of petroleum hydrocarbons, a prior extract cleanup step is not required. However, extract cleanup will increase the specificity of the analysis and may be included in the analysis procedure for a small additional cost (see Section 7.3.4.3). Without cleanup other fluorescing biogenic materials (pigments, phospholipids, etc.) may be detected and reported as petroleum contamination.

7.3.3.3 Biota Extraction

Homogenized tissue samples can be extracted wet using a combined polar:non-polar solvent or they can be dried and then extracted by any of the methods listed in Table III-3.13 of Volume I. Such methods result in substantial quantities of co-extracted biological material which interfere with most hydrocarbon determination techniques so that cleanup is required before analysis. It is more convenient to selectively extract the hydrocarbons from wet tissue with a non polar solvent. Drying and extraction are accomplished in one step by blending the thawed biota with pentane and anhydrous sodium sulfate (Gay et al., 1980). Although this is not the most exhaustive extraction procedure, it is a rapid and inexpensive method of extracting petroleum hydrocarbons from tissue samples.

As with sediments, extract cleanup is not required prior to UV-fluorescence detection of the petroleum hydrocarbons. Extract cleanup will increase the specificity of the analysis and may be included in the procedure if required (see Section 7.3.4.3). Potential interferences in the fluorescence detection are even greater with biota samples than with sediments.

7.3.4 Quantitative Analysis Procedures Providing Low Accuracy Determination of Petroleum Hydrocarbons

A rapid and inexpensive analysis can be performed on sediment and biota samples to estimate their level of petroleum hydrocarbon contamination. Procedures employing either UV-fluorescence or microgravimetric determination of the

petroleum hydrocarbons can be used for this purpose and are outlined in Tables 7.3.6 (for sediment) and 7.3.7 (for biota). The UV-fluorescence and the micrograviметric procedures are similar in cost and performance and method selection should be based on availability of equipment (i.e. fluorescence instrument or micrograviметric balance).

7.3.4.1 UV-Fluorescence Determination

This method is similar to that recommended above for screening samples for petroleum hydrocarbon content (Section 7.3.1). A rapid extraction is carried out and UV-fluorescence measurements are obtained directly on the extract without any prior cleaning or fractionation (Hoffman et al., 1979; Fong, 1976). However, in this case, the fluorescence response must be converted to petroleum concentrations.

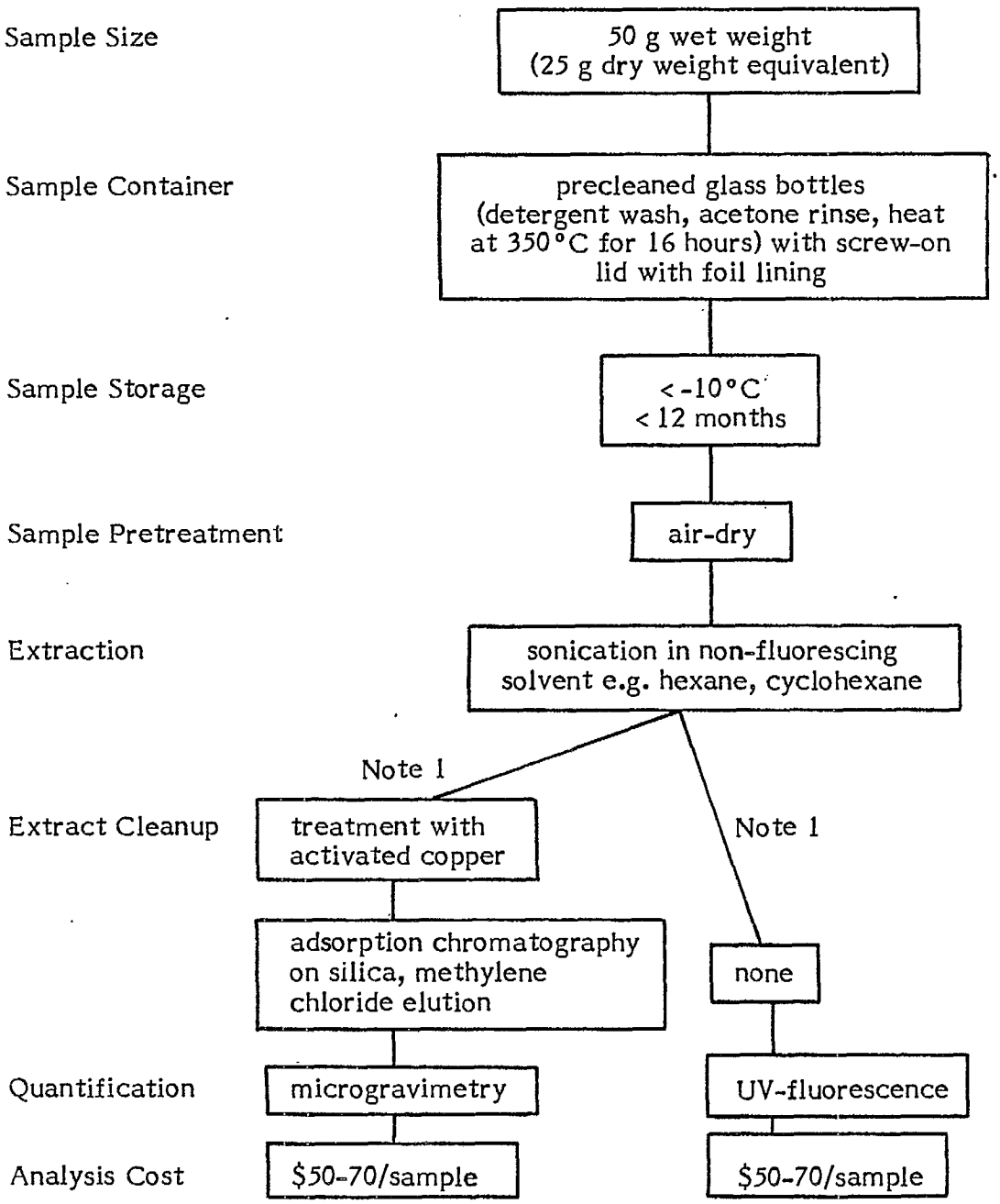
The key to quantification of petroleum compounds by UV-fluorescence spectroscopy is the choice of calibration standard. Ideally the standard should be identical in molecular composition to the petroleum in the samples, but this is very difficult with sediment and biota in which the petroleum may be fractionated or degraded or from a variety of sources. An arbitrary reference oil may be used to calibrate the UV-fluorescence response and the results reported as "equivalent oil concentrations". This procedure is most useful in cases in which a target oil is available, i.e., in oil spill situations, but is not commonly used otherwise.

An alternative method is the use of a chrysene solution for calibration and the results reported as "chrysene equivalents" (Cretney and Wong, 1974). Results using these calibration methods may not necessarily agree with each other or with results by more accurate quantification techniques. This may be especially true for sediment and biota extracts in which the petroleum could be variably fractionated. Quantitative analysis using a UV-fluorescence technique is therefore most useful when results are to be used only for comparison with other results by the same method and when absolute petroleum concentrations are not required.

The use of a rigorously quantified sediment or biota extract as the calibration standard is potentially more accurate than either of these calibration techniques and may be useful when estimations of absolute concentrations are desired or when variable fractionation of the petroleum is suspected. The analysis procedures required to rigorously quantify such a standard extract (exhaustive cleanup of the extract followed by micrograviметric or GC/FID quantification) are described in Sections 7.3.5 and 7.3.6.

Table 7.3.6

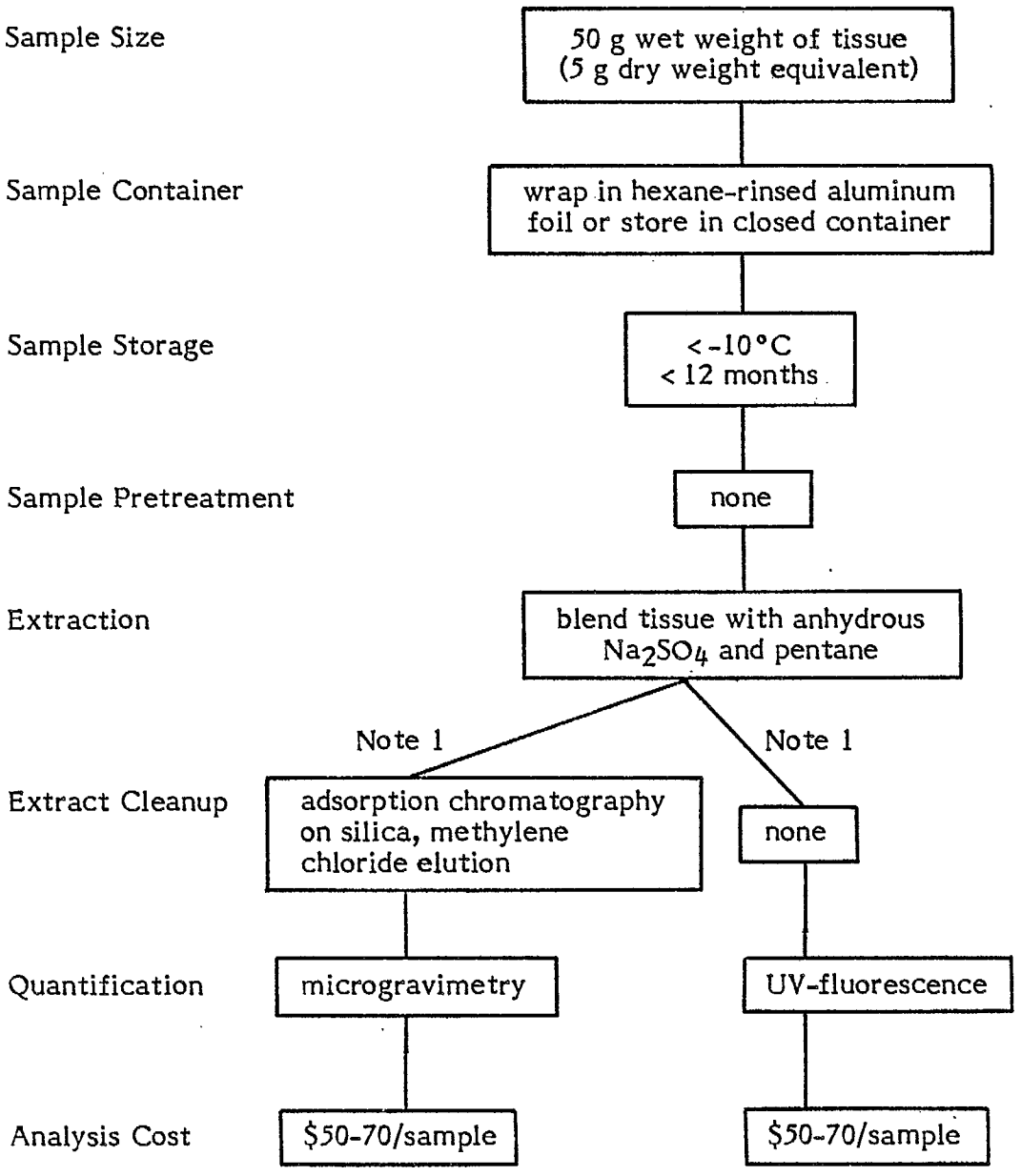
Low Accuracy Determination of Petroleum Hydrocarbons in Sediments



Note 1: The UV-fluorescence method is more specific for petroleum hydrocarbons than the micrograviметric method but use of the micrograviметric method avoids the calibration problems associated with UV-fluorescence method.

Table 7.3.7

Low Accuracy Determination of Petroleum Hydrocarbons in Biota. Estimation of Contamination Levels



Note 1: The UV-fluorescence method is more specific for petroleum hydrocarbons than the microgravimetric method but use of the microgravimetric method avoids the calibration problems associated with UV-fluorescence method.

7.3.4.2 Microgravimetric Determination

As with methods employing a UV-fluorescence determination of the petroleum hydrocarbons, a rapid and inexpensive extraction technique is selected when microgravimetric quantification is employed. However, in this case cleanup of the extract solution is required to ensure some specificity of the analysis for petroleum hydrocarbons. This increases the analysis time and cost slightly but avoids the problems associated with calibration of the UV-fluorescence response. The microgravimetric method is also susceptible to error due to loss of volatile petroleum components or contamination of the sample by dust, and it is less specific towards petroleum than UV-fluorescence. The use of microgravimetry is described by Clark and Brown (1977).

7.3.4.3 Sediment Extraction and Extract Cleanup

It is recommended that pre-dried sediment be extracted by sonication in cyclohexane (Tan, 1979) when a low accuracy analysis is being carried out. The rationale for selection of this method is discussed in Section 7.3.3.2.

UV-fluorescence measurements can be obtained directly on this extract solution. If greater specificity in the UV-fluorescence measurement is desired, or if the petroleum hydrocarbons are determined by microgravimetry, some prior cleanup of the extract is required.

A cleanup technique is required to separate co-extracted polar lipid material from the petroleum hydrocarbons. Although several techniques have been used for this purpose, adsorption chromatography on a silica column using methylene chloride:pentane for elution is recommended (Tan, 1979). It is a rapid and inexpensive technique and provides reasonable cleanup of extract solutions with good recovery of petroleum hydrocarbons. Loss of some petroleum hydrocarbons can be experienced if alumina or florisil columns are used and they should be avoided in quantitative analysis. The use of a gel column such as Sephadex LH20 will provide only a partial cleanup of the extract (it is normally used in addition to silica cleanup to separate lipids and provide fractionation of the petroleum into non-polar and aromatic fractions. See Section 7.3.8).

Elemental sulfur must be removed from sediment extracts prior to microgravimetric determinations. This is accomplished by treatment of the extract solution with activated copper powder (Blumer, 1957). This treatment is not required prior to UV-fluorescence measurement (or for biota extracts).

7.3.4.4 Biota Extraction and Extract Cleanup

It is recommended that biota tissue be extracted by blending with pentane and anhydrous sodium sulfate (Gay et al., 1980). The rationale for selection of this method is discussed above in Section 7.3.3.3.

UV-fluorescence measurements are made directly on this extract solution. Cleanup of the extract is required prior to microgravimetric determinations. As discussed above for sediment extracts (Section 7.3.4.3) adsorption chromatography on silica using methylene chloride:pentane for elution is the recommended method (Tan, 1979). No treatment for sulfur removal is required.

7.3.5 Quantitative Analysis Procedures Providing Moderate Accuracy

Analytical methods of at least moderate accuracy are required to monitor more subtle changes in petroleum contamination levels in sediment and biota samples. Suitable methods are those which are relatively insensitive to variations in petroleum composition. In certain situations, the added requirement of low detection limit may be necessary. The recommended analysis procedure for sediment samples is outlined in Table 7.3.8 and the procedure for biota in Table 7.3.9.

7.3.5.1 Determination of Petroleum Hydrocarbons

As indicated in Table 7.3.1, several techniques are sufficiently accurate to monitor trends in petroleum concentrations in extract solutions. These are ultraviolet spectroscopy and UV-fluorescence, microgravimetry and gas chromatography with either flame ionization or mass spectral detection. Both the ultraviolet and the UV-fluorescence responses are difficult to calibrate accurately and neither technique is recommended for use when the petroleum composition is variable due to fractionation or degradation (see discussion in Section 7.3.4.1 and in Sections III-3.4.6 of Volume 1). Therefore either a gas chromatographic or a microgravimetric quantitation step is preferred in this situation. These two techniques are comparable in accuracy but microgravimetry is less sensitive than either GC/FID or GC/MS. However, microgravimetry is less expensive and is recommended for use when its higher detection limit (10 $\mu g \cdot g^{-1}$) is acceptable. A GC method is recommended when lower detection limits (1 $\mu g \cdot g^{-1}$) are required. GC/FID

Table 7.3.8

Moderate Accuracy Determination of Petroleum Hydrocarbons in Sediments

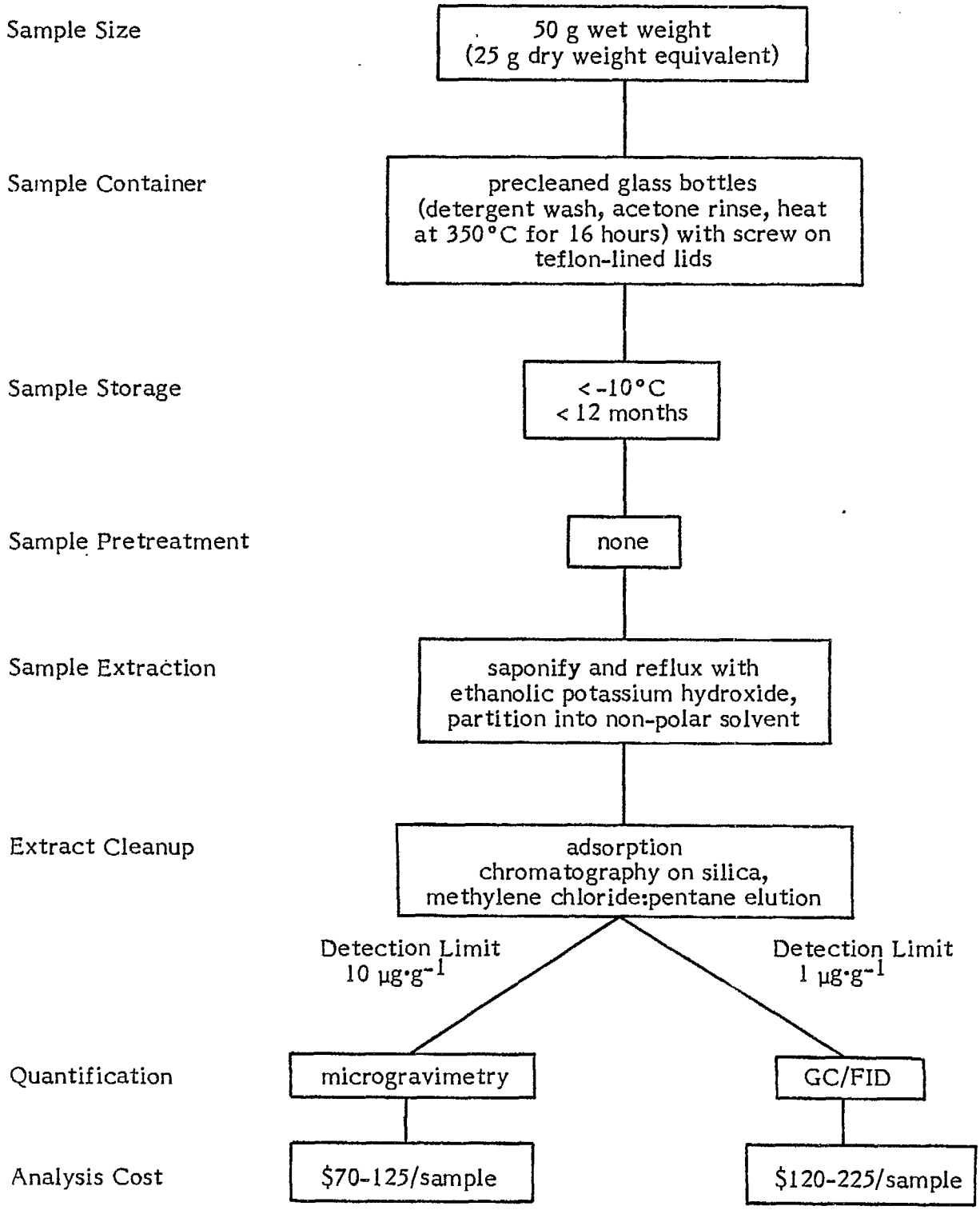
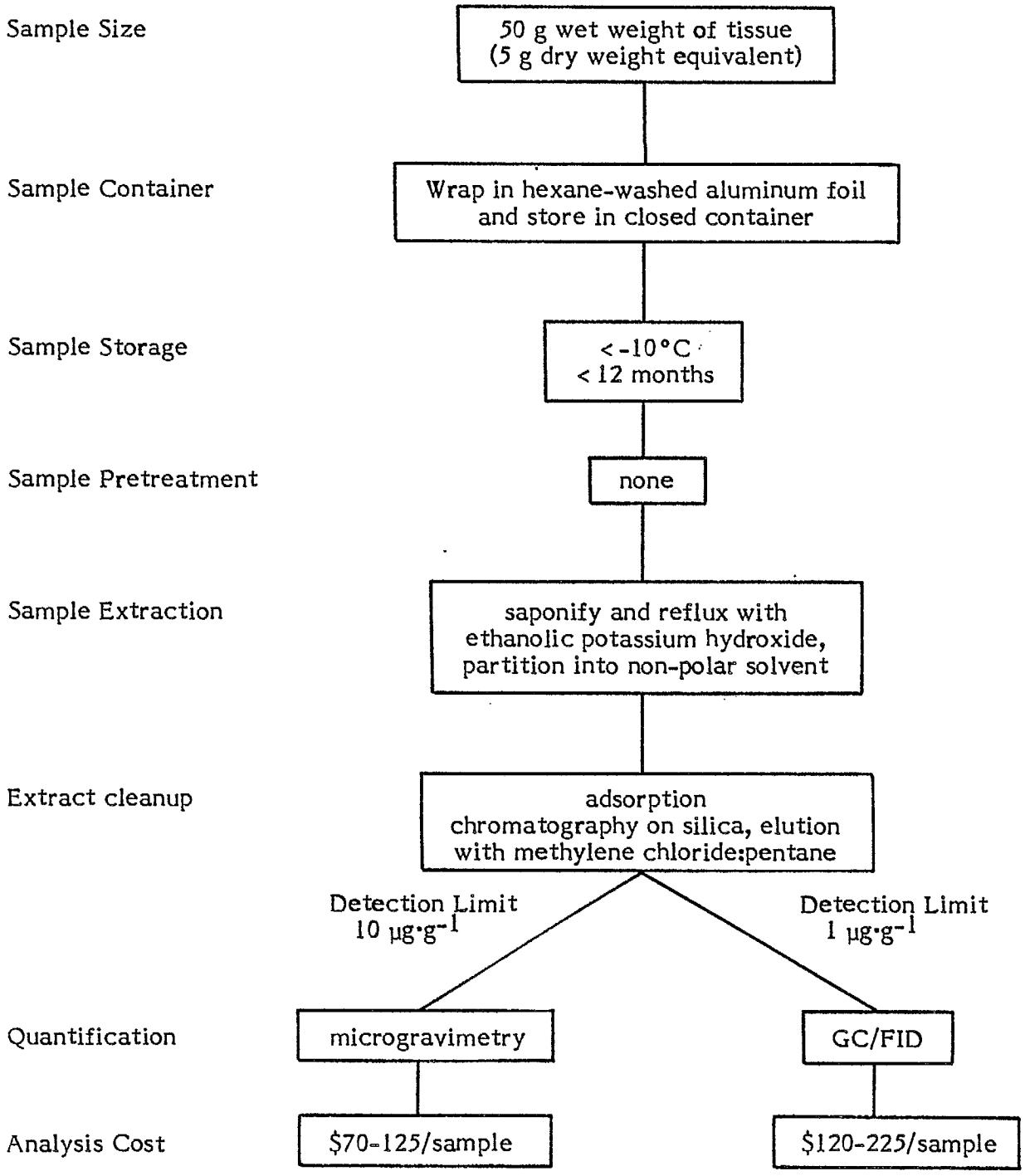


Table 7.3.9

Moderate Accuracy Determination of Petroleum Hydrocarbons in Biota



is the technique of choice in this case as it is less expensive, more available and easier to calibrate for quantitative work than GC/MS.

7.3.5.2 Sediment Extraction and Extract Cleanup

When monitoring changes in petroleum contamination levels in samples, an efficient extraction method yielding precise results is required. Although many of the extraction procedures described in Section III-3.4.4.1 of Volume 1 exhibit high recovery of petroleum hydrocarbons, the best precision has been reported when the sediment was saponified before extraction (Wong and Williams, 1980). This procedure (reflux with ethanolic potassium hydroxide, partition into a non-polar solvent) is described in detail by Plumb (1981) and Cretney et al. (1980) and is recommended for accurate quantitative analysis. It has the additional advantages of: 1) not requiring pre-dried sediments, 2) resulting in partially cleaned extract solutions, and 3) eliminating the need for a subsequent sulfur removal step.

Cleanup of the extract solution is required prior to quantification of the petroleum hydrocarbons. Silica adsorption chromatography and gel permeation chromatography on Sephadex LH20 both provide effective clean-up methods. For total hydrocarbon measurements, with no fractionation of hydrocarbons, a silica column eluted with methylene chloride:pentane will provide an adequate cleanup (Tan, 1979). For compound separation into non-polar and aromatic fractions, a compound method using Sephadex LH20 and silica columns is recommended (Giger and Schaffner, 1978; see Section 7.3.6). Low recoveries of some aromatics can occur with alumina or florisil columns. Their use is not recommended therefore for quantitative analyses.

7.3.5.3 Biota Extraction and Extract Cleanup

Saponification of the tissue by reflux with ethanolic potassium hydroxide, followed by partition of the hydrocarbons into a non polar solvent (Cretney et al., 1980) is the recommended extraction technique for this analysis. No prior drying or other treatment of the sample is then required. Although saponification is a slightly less efficient extraction technique than a soxhlet extraction, it is more rapid and the accompanying solvent partition step eliminates much of the polar lipid material which could interfere in the subsequent quantification step (see discussion in Section III-3.4.4.2 of Volume 1).

The cleanup, fractionation and quantification steps of biota extracts produced in this manner are identical to those required for sediment extracts. As discussed in Section 7.3.5.2, adsorption chromatography on silica columns is the most appropriate cleanup procedure when determining the total hydrocarbon content of extract solutions. Fractionation of the cleaned extract is not carried out unless compositional information is also required. In such a case the aliphatic and aromatic fractions of the extract obtained from Sephadex LH20 columns (see Section 7.3.8) can be cleaned and quantified separately.

7.3.6 Quantitative Analysis Procedures Providing High Accuracy, High Sensitivity Determination of Petroleum Hydrocarbons

An analytical method of high accuracy and high sensitivity is required to monitor baseline levels of petroleum hydrocarbons in sediment and biota samples. The utmost care is required in sample handling and the most rigorous extraction and extract cleanup procedures are carried out. The most accurate and sensitive quantification technique is used. The recommended analysis procedure for sediment samples is outlined in Table 7.3.10 and the procedure for biota in Table 7.3.11.

7.3.6.1 Determination of Petroleum Hydrocarbons

As indicated in Table 7.3.1, only gas chromatographic techniques (either GC/FID or GC/MS) have sufficient sensitivity to accurately measure the low levels of petroleum hydrocarbons expected in arctic background samples. A detection limit of 1 $\mu g \cdot g^{-1}$ can be achieved. The microgravimetric technique is also accurate but is useful only for samples containing higher levels of petroleum contamination (> 10 $\mu g \cdot g^{-1}$). GC/FID is recommended for quantification of petroleum hydrocarbons as it is less expensive, more available and easier to calibrate for quantitative work (Farrington and Tripp, 1975). However, GC/MS can be used if compositional information is also required (see Section 7.3.8) and is preferred for the analysis of polycyclic aromatic hydrocarbons (Tan, 1979; Shaw, 1979).

7.3.6.2 Sediment Extraction and Extract Cleanup

A rigorous extraction procedure is required to completely extract petroleum hydrocarbons from sediment. Saponification of the sediment sample (by reflux in

Table 7.3.10

High Accuracy, High Sensitivity Determination of Petroleum Hydrocarbons in Sediments

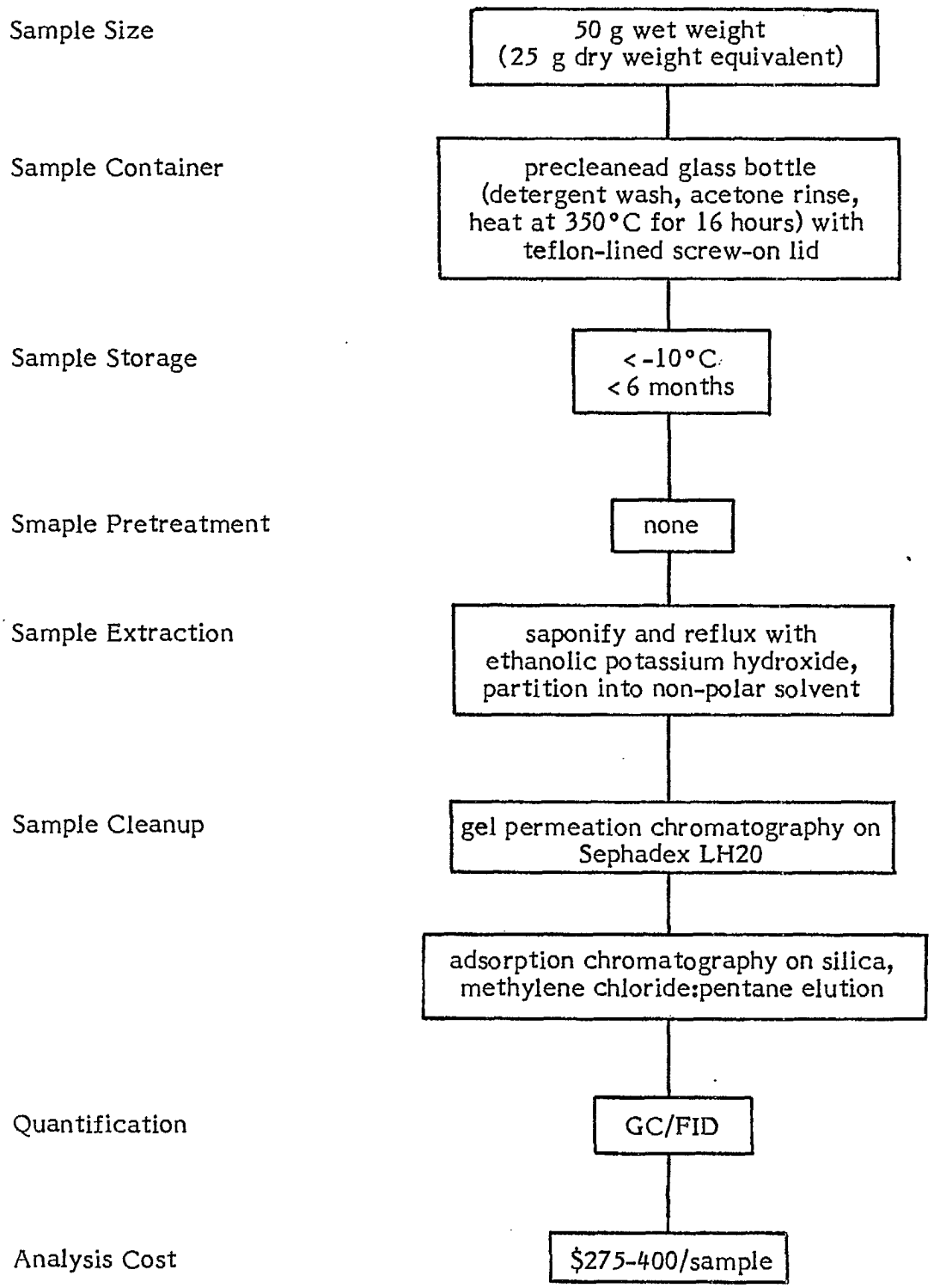
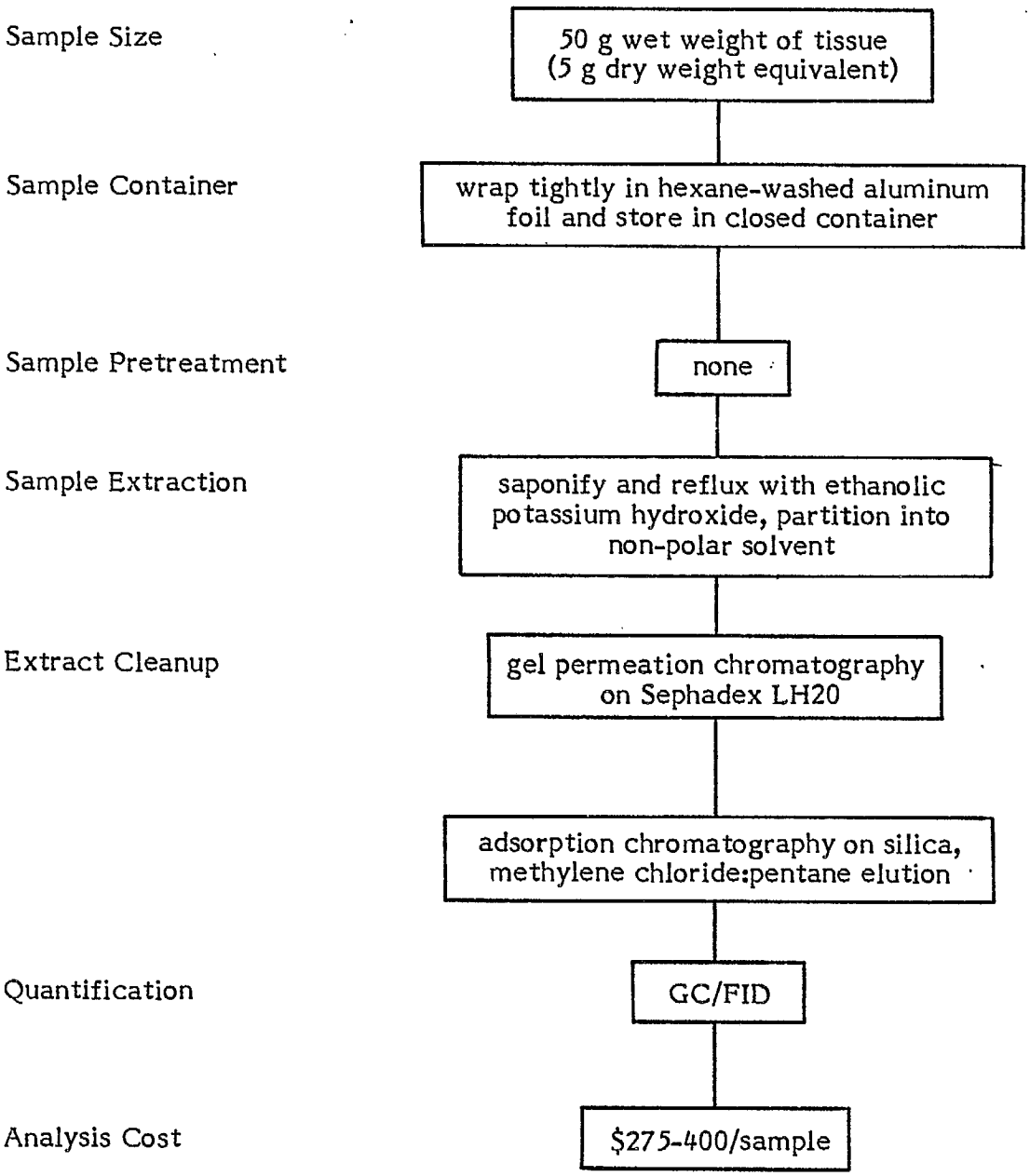


Table 7.3.11

High Accuracy, High Sensitivity Determination of Petroleum Hydrocarbons in Biota



ethanolic potassium hydroxide and partitioning into a non-polar solvent) is reported to result in high recoveries of petroleum hydrocarbons, with good precision and minimal interference from polar co-extracted material. This is the recommended extraction procedure - it is described by Plumb (1981) and by Cretney et al. (1980). Other extraction procedures may result in incomplete recovery of petroleum hydrocarbons (e.g., ball mill tumbling methods) or produce extracts that include high levels of co-extracted material and require a more thorough cleanup (e.g., soxhlet methods).

Cleanup of the extract is required prior to the quantification step to remove interfering co-extracted material. The choice of extract clean up and separation method depends on the intended goal and the instrumental technique to be used. However, for the analysis of petroleum hydrocarbons at baseline levels, the fossil fuel hydrocarbons must be separated from interfering hydrocarbons such as those of recent biological origin and higher molecular weight lipids. This is desirable even if the technique normally does not require any type of separation, such as a total hydrocarbon determination by gas chromatography. The method of choice for this situation is gel permeation chromatography on Sephadex LH20 followed by silica chromatography (Giger and Schaffner, 1978). Adsorption chromatography on alumina or Florisil may result in loss of petroleum hydrocarbons and should be avoided.

7.3.6.3 Biota Extraction and Extract Cleanup

Extraction of biota tissue by saponification (reflux in ethanolic potassium hydroxide, partitioning into a non-polar solvent (Cretney et al., 1980)) is the recommended procedure. No prior drying or other treatment of the sample is then required. Although saponification is a slightly less efficient extraction technique than a soxhlet extraction, it is more rapid and the resultant extract contains fewer potential interferences to the quantification step.

The recommended cleanup procedure is that of Giger and Schaffner (1978) - size separation by gel permeation chromatography on Sephadex LH20 followed by polarity separation by adsorption chromatography on silica. The rationale for this selection is given in Section 7.3.5.2 above.

7.3.7 Quantitative Analysis Procedures for the Determination of Low Molecular Weight Petroleum Hydrocarbons in Sediment and Biota

Because of their volatility, low molecular weight hydrocarbon compounds (those containing less than approximately eleven carbon atoms) in sediment and biota

are not amenable to analysis by conventional solvent extraction techniques. However, specialized technqiues based on gas chromatography are available to isolate and quantitate these compounds. Either static headspace sampling or dynamic headspace purging of the sample can be used, followed by a gas chromatographic identification and quantification of the hydrocarbons. The static headspace technique requires no special equipment and is therefore less expensive and more available than the dynamic headspace purge. However, the static technique is an equilibrium technique (the extraction is not taken to completion) and is potentially subject to many sources of error even when elaborate calibration is carried out. Use of this technqiue should be restricted to situations in which only semi-quantitative results are required.

The dynamic headspace technique (Michael et al., 1980) is potentially more accurate as it causes the equilibrium to be pushed toward completion resulting in almost complete extraction of the volatile hydrocarbon components. However a special apparatus is required, thus limiting the availability of the analysis. This is a recommended procedure, however, if quantitative results are required.

7.3.8 Qualitative Analysis of Petroleum Hydrocarbons in Sediment and Biota Samples (Compositional Information)

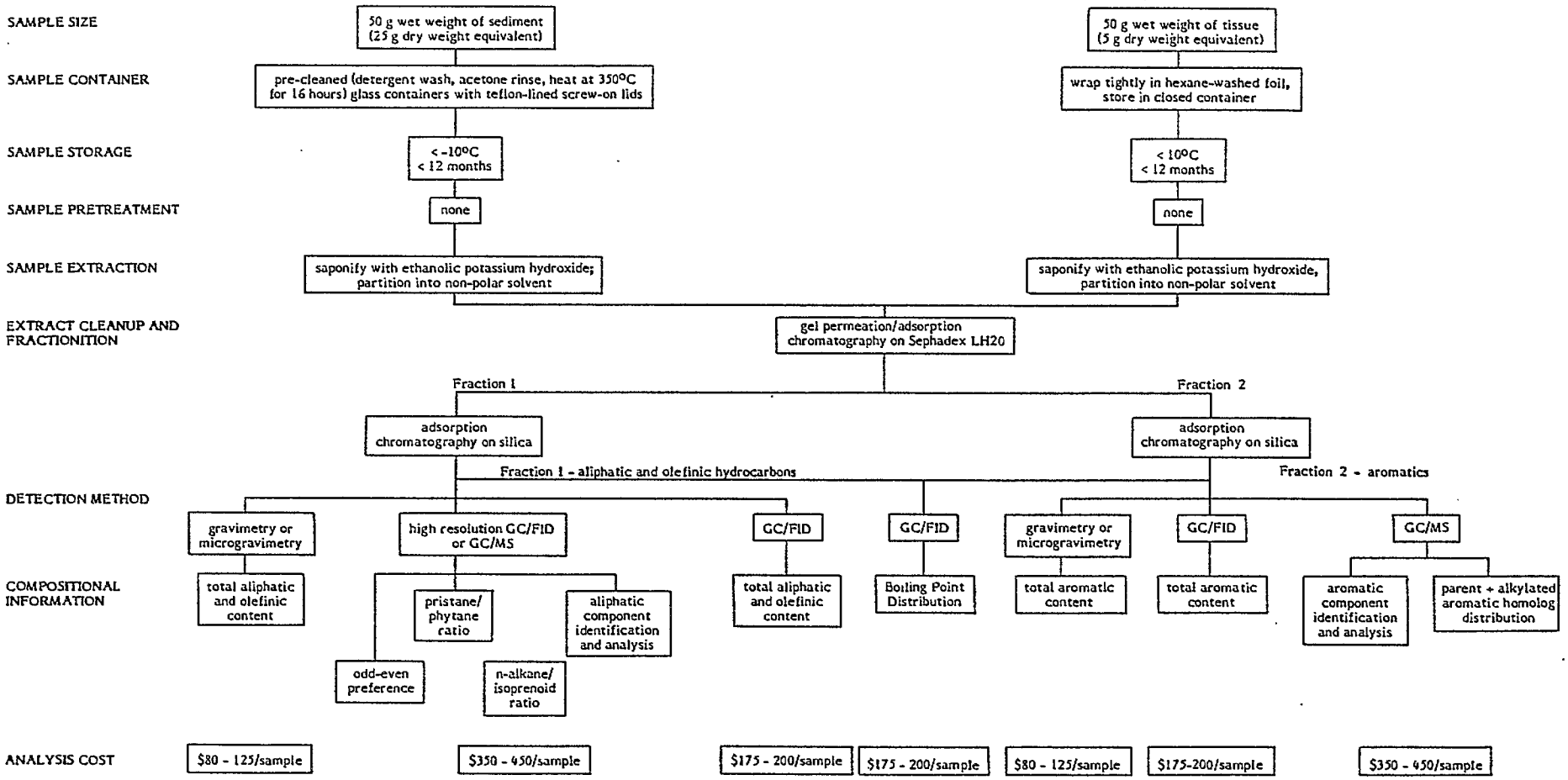
The types of compositional information which can be determined on the petroleum hydrocarbons extracted from sediment and biota samples are listed in Table 7.3.3. For these analyses, careful sample handling is required to avoid loss of hydrocarbon components or contamination of the sample. An efficient extraction followed by rigorous cleanup and fractionation of the extract is required. The procedures for compositional analysis of sediments and biota tissue are outlined in Table 7.3.12.

The saponification extraction recommended above for sediment and tissue (see Sections 7.3.6.2 and 7.3.6.3) is also useful here. This method provides efficient recovery of petroleum hydrocarbons from both sediment and biota samples while yielding moderately clean extract solutions that do not cause interference in the instrumental analysis.

Extract cleanup and fractionation are required prior to compositional analyses of the extracted petroleum. Adsorption chromatography on Florisil or on silica or silica/alumina columns and adsorption/size separation on Sephadex LH20 are commonly used. Low recoveries of some aromatics can be experienced with Florisil

Table 7.3.12

Compositional Analysis of Petroleum Hydrocarbons in Sediments and Biota



and alumina columns and they are not recommended for petroleum determinations. Chromatography on silica is required to adequately clean the extract (remove esters) while chromatography on Sepadex LH20 is required to accurately fractionate the sample into aliphatic (non-polar) and aromatic portions. Chemical separation of aromatics by complexation with dimethylsulfoxide or trinitrofluorenone has been used in some methods. Such fractionation is useful when only the concentration of aromatics in the sample is of interest. No other compositional information can be obtained and there does not appear to be any advantage to these methods over Sepadex LH20 fractionation even for simple quantification of the aromatic fraction. **Therefore the cleanup and fractionation procedure of Giger and Schaffner (1978) is recommended whereby the sample is fractionated on a Sepadex LH20 column and the two fractions are cleaned on silica columns to remove non-petroleum contaminants.**

The instrumental technique used for analysis of the aromatic and aliphatic fractions depends on what compositional information is required. GC/FID and GC/MS are most commonly used (Farrington and Tripp, 1975; Shaw, 1979; Tan, 1979). Depending on the goal, quantitative analysis of the aliphatic (non-polar) fraction can be carried out by the techniques recommended in Sections 7.3.4.2, 7.3.5.1 or 7.3.6.1 (gravimetry or gas chromatography). Quantitative analysis of the aromatic fraction can be carried out by the techniques recommended in Sections 7.3.4.1, 7.3.4.2, 7.3.5.1 and 7.3.6.1 above (gravimetry, gas chromatography or UV-fluorescence).

The boiling point distribution (or distillation curve) of the extracted petroleum is also obtained by GC/FID. The aliphatic and aromatic fractions can be recombined prior to this analysis, or analyzed separately.

Several parameters can be determined from the aliphatic fraction of the extract. These include odd-even preference, pristane/phytane ratio and n-alkane/isoprenoid ratio. High resolution (capillary column) GC/FID is required for these analyses. These parameters can also be measured on the unfractionated extract (i.e., before the Sephadex LH20 fractionation) but the results by GC/FID would be less accurate due to a greater possibility of peak overlap.

The aromatic fraction of the extract can be analyzed by GC/MS to provide both a parent aromatic compound distribution and an alkylated aromatic homolog distribution. This information can be obtained by no other technique.

GC/MS is the ideal technique to use for both major and minor aromatic component identification. Major components may be identifiable by high resolution (capillary column) GC/FID, but with less certainty. Major component concentrations can be determined by GC/FID or GC/MS, but minor components must be analyzed by GC/MS.

7.4 Oil and Grease

Oil and grease is a procedurally-defined quantity dependent on the organic solvent and technique used for extraction and on the quantification method employed. Three standard methods exist (Inland Waters Directorate, 1979; EPA (Plumb, 1981) and APHA, AWWA, WPCF, 1980). The various procedures used for oil and grease determinations on sediments are reviewed in Section III-3.5, Volume 1 and discussed below.

7.4.1 Sample Requirements

Approximately 20 g (wet weight) of sediment is required for oil and grease determinations. Precleaned (solvent-rinsed) metal containers or glass jars with teflon-lined lids can be used for sample storage. Sediments are acidified and maintained at -10°C (Inland Waters Directorate, 1979).

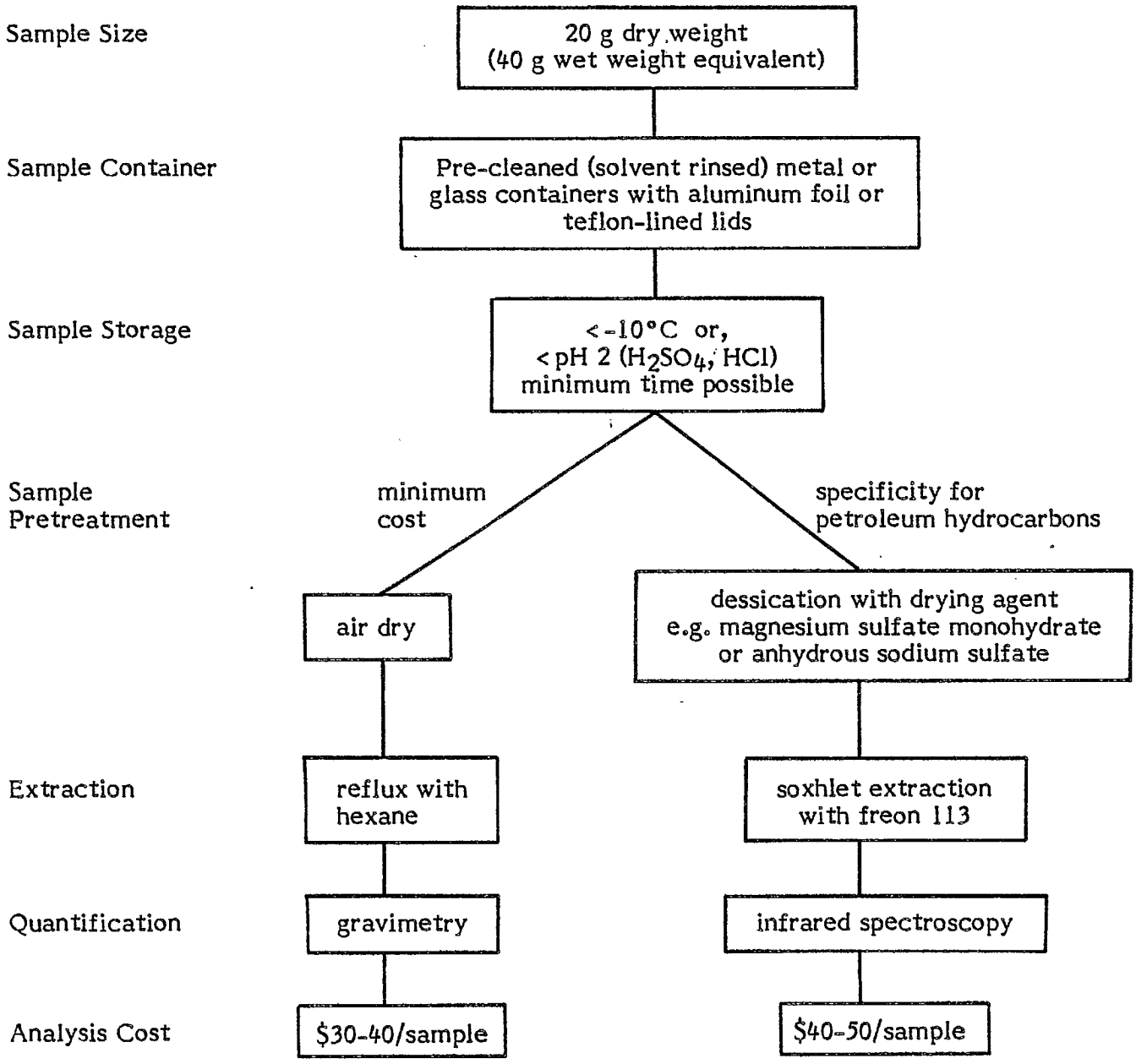
7.4.2 Analysis Procedures

Selection of a method for oil and grease determinations can be based on considerations of cost and accuracy. Variations in the analysis cost will be due essentially to the method chosen for quantification - gravimetry or infrared spectroscopy. A gravimetric (or microgravimetric) based method will be less expensive than an infrared method. However, infrared spectroscopy is more specific to petroleum hydrocarbons while elemental sulfur in the sediment can cause significant error in gravimetric methods. **Therefore a gravimetric based method (Inland Waters Directorate, 1979; Plumb, 1981; APHA, AWWA, WPCF, 1980) is recommended as a low cost method and an infrared method (Plumb, 1981) is recommended as a more petroleum hydrocarbon specific method.** These methods, outlined in Table 7.4.1, will provide comparable detection limits.

For maximum accuracy the sediment should not be air-dried to prevent loss of volatile hydrocarbon material. The sample should be dessicated by grinding with magnesium sulfate or anhydrous sodium sulfate (Plumb, 1981). Soxhlet extraction is recommended in all three standard methods. Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane) is used as the extraction solvent as it does not interfere with the infrared quantification. Calibration of the infrared absorbance requires the use of standard solutions and is discussed in detail by Plumb, 1981.

Table 7.4.1

Determination of Oil and Grease in Sediments



A less expensive oil and grease determination, using a gravimetric or micrograviметric measurement of the extractable material, would not require a soxhlet extraction. Reflux extraction of an air-dried sample is often used for speed and convenience (Thomas et al., 1982). Freon (1,1,2-trichloro-1,2,2 trifluoroethane) can be used as the extraction solvent but hexane (Thomas et al., 1982) or petroleum ether (Inland Waters Directorate, 1979) are also suitable. Hexane is commonly used for this purpose and results reported as "Hexane Extractables". The oil and grease or hexane extractables are determined from the weight of non-volatile (room temperature) material in an aliquot of the extract (Inland Waters Directorate, 1979; Plumb, 1981).

7.5 Phenols

The term phenols refers to the range of compounds which have a hydroxyl functional group on an aromatic nucleus. There are two approaches to the determination of environmental phenols: the application of **procedurally-defined** methods for "total phenols" and the determination of **individual compounds**. Methods for performing both these types of analyses on sediment and biota samples are reviewed in Section III-3.6 of Volume 1 and discussed below.

7.5.1 Sample Requirements

Approximately 50 g (wet weight) of sediment (Plumb, 1981) or 25 g (wet weight) of tissue (Allen and Sills, 1974) is required for phenols analysis. Sediment samples should be stored in glass containers with teflon-lined lids at 4 C° in the dark (Plumb, 1981). Biota samples should be wrapped tightly in foil and stored in closed containers (Allen and Sills, 1974). The maximum recommended storage time (24 hours; Plumb, 1981) may not be possible for samples collected in the Arctic but analyses for phenols should be carried out as soon as possible. Sediment samples are analyzed wet to minimize loss of phenolic compounds (Plumb, 1981). Biota tissue is pre-dried by homogenization with anhydrous sodium sulfate (Allen and Sills, 1974).

7.5.2 Determination of Total Phenols

Analytical methods for "total phenols" are screening procedures which are sensitive to the general phenol structure. No differentiation among the various phenols is available and all phenolic compounds are reported as "phenol equivalents". Most methods involve a colorimetric quantification step following conversion of the phenolic compounds to a coloured dye complex. Some phenols are non-reactive to this conversion and the molar absorptivity of the dye complex is maximum for phenol itself and may be less for substituted phenols. Thus the results of colorimetric determinations of total phenols represent a minimum estimate. Improved accuracy is obtained if a gas chromatographic quantification is performed (see Section 7.5.2 below on the determination of individual molecules). However "total phenols" is a procedurally dependent quantity and meaningful results can only be achieved if obtained in the context of standardized procedures.

The only standard procedure for total phenols in sediments is that of the EPA (Plumb, 1981) and it is therefore recommended for use. No standard method is available for phenols in biota tissue but it is suggested that a literature method for extracting individual phenol compounds from tissue be used. After cleanup of the tissue extract to remove fats and fat-soluble compounds, the total phenols content can be determined by the EPA procedure for sediment extracts. **The recommended analytical procedures are outlined in Table 7.5.1 for "total phenols" in sediment and in Table 7.5.2 for biota.**

7.5.2.1 Extraction

a) Sediment

In the EPA standard procedure the extraction of phenols from sediments is accomplished by steam distillation (Plumb, 1981). Some literature analyses of specific phenolic compounds recommend a soxhlet extraction of the sediment, however this would result in higher recoveries of the less volatile phenol molecules[1]. Therefore total phenol levels from a soxhlet extraction method would not be comparable to results from the standard EPA methods.

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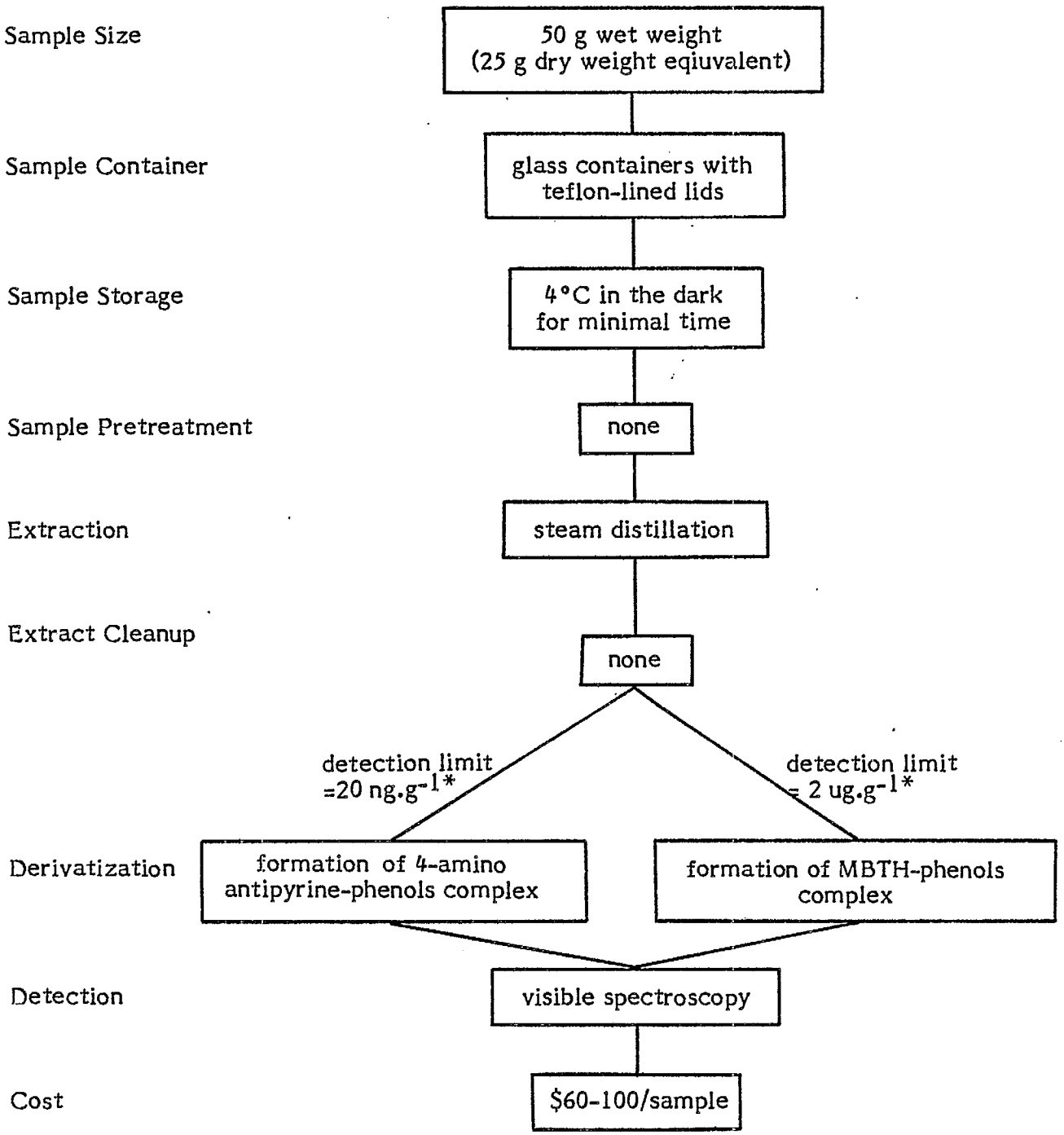
[1] The EPA standard method using steam distillation is adapted from standard methods for water analysis. Water samples (not expected to contain high levels of these less volatile phenols) are not subjected to soxhlet extraction.

Table 7.5.1

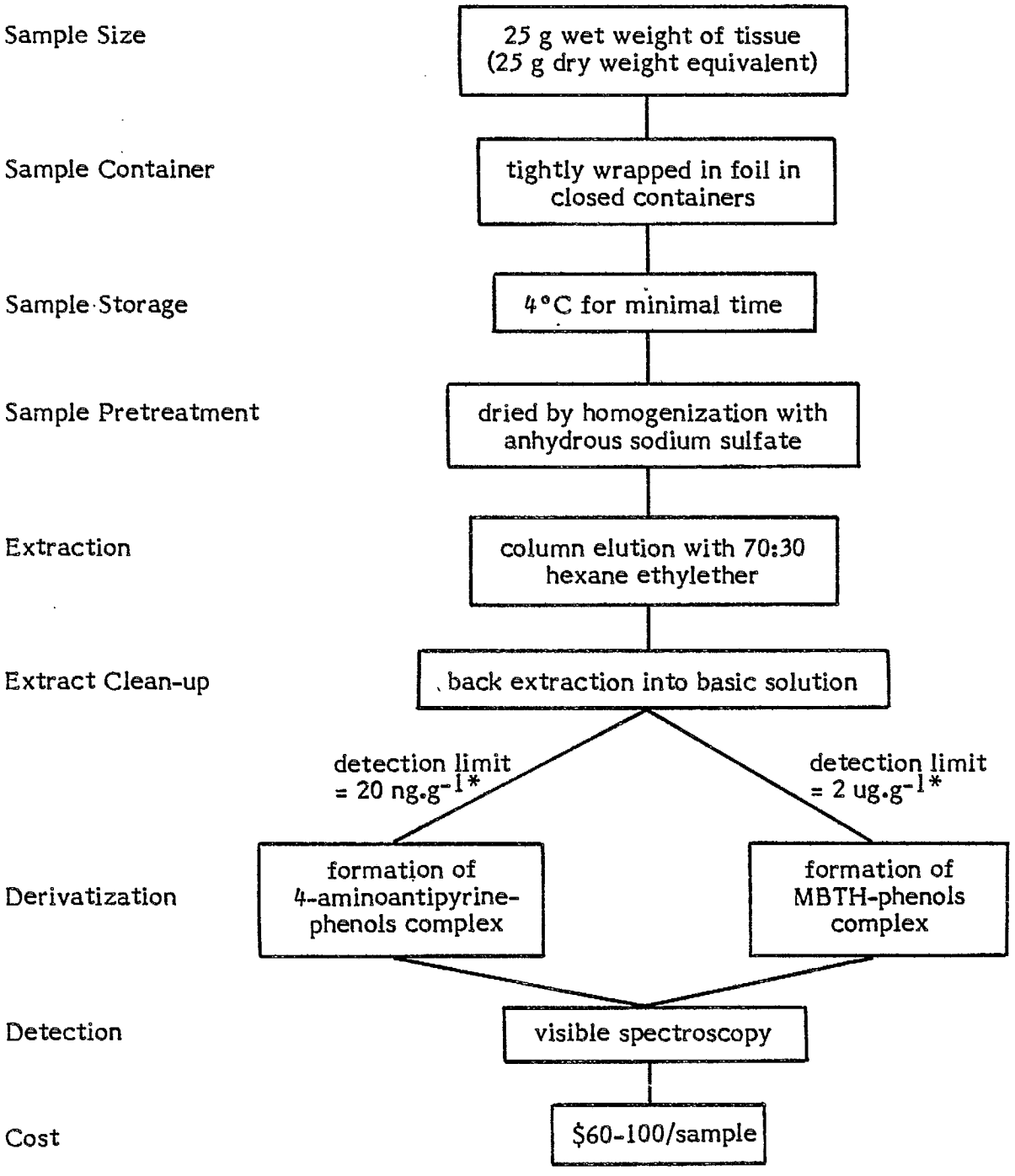
Determination of Total Phenols in Sediments



* Based on 500 mL distillate, 25 g sediment (dry weight)

Table 7.5.2

Determination of Total Phenols in Biota



*Based on 100 mL distillate, 5 g tissue (dry weight)

b) Biota

The recommended procedure for extracting phenolic compounds from tissue uses a 70:30 hexane:ethyl ether mixture and column elution followed by cleanup via back extraction into basic solution (Allen and Sills, 1974). This method is reported to give excellent extraction recoveries and is simple to perform. A second literature procedure (Hattula et al., 1978) for extraction of phenols from biota does not report recoveries and requires a thin layer chromatographic step to separate the phenols from co-extracted material.

7.5.2.2 Quantification Techniques

The extracted phenols can be determined by spectrofluorimetry, by ultraviolet spectroscopy or by visible spectroscopy (colorimetry) after conversion to a 4-aminoantipyrine or 3-methyl-2-benzothiazolinone hydrazone-HCl (MBTH) dye-complex. The standard EPA method for "total phenols" in sediments uses a colorimetric quantification step. Two procedures are given: the formation and measurement of the 4-aminoantipyrine-phenols complex and the formation and measurement of the MBTH-phenols complex. No criteria are given to choose between the two procedures, but the 4-aminoantipyrene method has a lower detection limit (20 $ng.g^{-1}$ assuming 25 g sediment and 500 mL distillate) than the MBTH method (2 $\mu g.g^{-1}$). Both methods use phenol as the calibration standard and results are reported as phenol equivalents. In both cases the molar absorptivity of the dye complex is maximum for phenol itself and may be less for substituted phenols. In addition, the MBTH method detects phenol compounds which do not react with the 4-aminoantipyrine reagent. Thus "total phenols" by these two methods may not be comparable and will give results that are lower than the concentration of "true" phenols. **It is recommended that the MBTH colorimetric determination be used whenever possible and that the 4-aminoantipyrine colorimetric determination be used only when its lower detection limit is required.**

Fluorimetric and UV methods for the determination of "total phenols" are rapid and do not require derivatization of the phenolic compounds. Their main disadvantage is the low molar absorptivity of most phenols as compared to phenol itself. Thus high levels of substituted phenols could be reported as low levels of phenol equivalents. Fluorimetric and UV measurements are also not specific to the phenol structure: other aromatic compounds (including some petroleum hydrocarbons)

would be detected and reported as phenols. Results by either of these methods would not be comparable to results by the standard EPA colorimetric methods[1]. The UV and fluorimetric methods are simpler to perform and less expensive then the derivatization-colorimetric procedures but are useful only when other aromatic contaminants are known to be absent and when results are to be compared only with analyses by the same procedure.

7.5.3 Determination of Individual Phenol Compounds

No standard methods exist for the determination of individual phenol compounds in sediment or biota samples. A few procedures are reported in the literature for specific phenol compounds and each of these employs its own extraction and extract cleanup procedure followed by separation and quantification of the phenols. The recommended procedures for determining individual phenols in sediment and biota are outlined in Table 7.5.3. Methods for the determination of chlorophenols (a subset of the phenols group) are discussed in Section 7.6.

7.5.3.1 Extraction

a) Sediment

The steam distillation method used for the extraction step in "total phenols" determinations on sediment samples does not extract all phenols and is not recommended for individual compound determinations. Three solvent extraction procedures are reported in the literature for specific compounds. **The method of Jungclaus et al. (1978) (soxhlet extraction with isopropanol) has been used for the widest range of compounds and is suggested for general use.** The resultant extract solution is analyzed without cleanup. If co-extracted non-polar hydrocarbons (e.g., alkanes) interfere with the determination of the phenols they can be separated by adsorption chromatography on silica (Games and Hites, 1977) but this step should be avoided if possible as it could result in loss of some phenol material on the silica.

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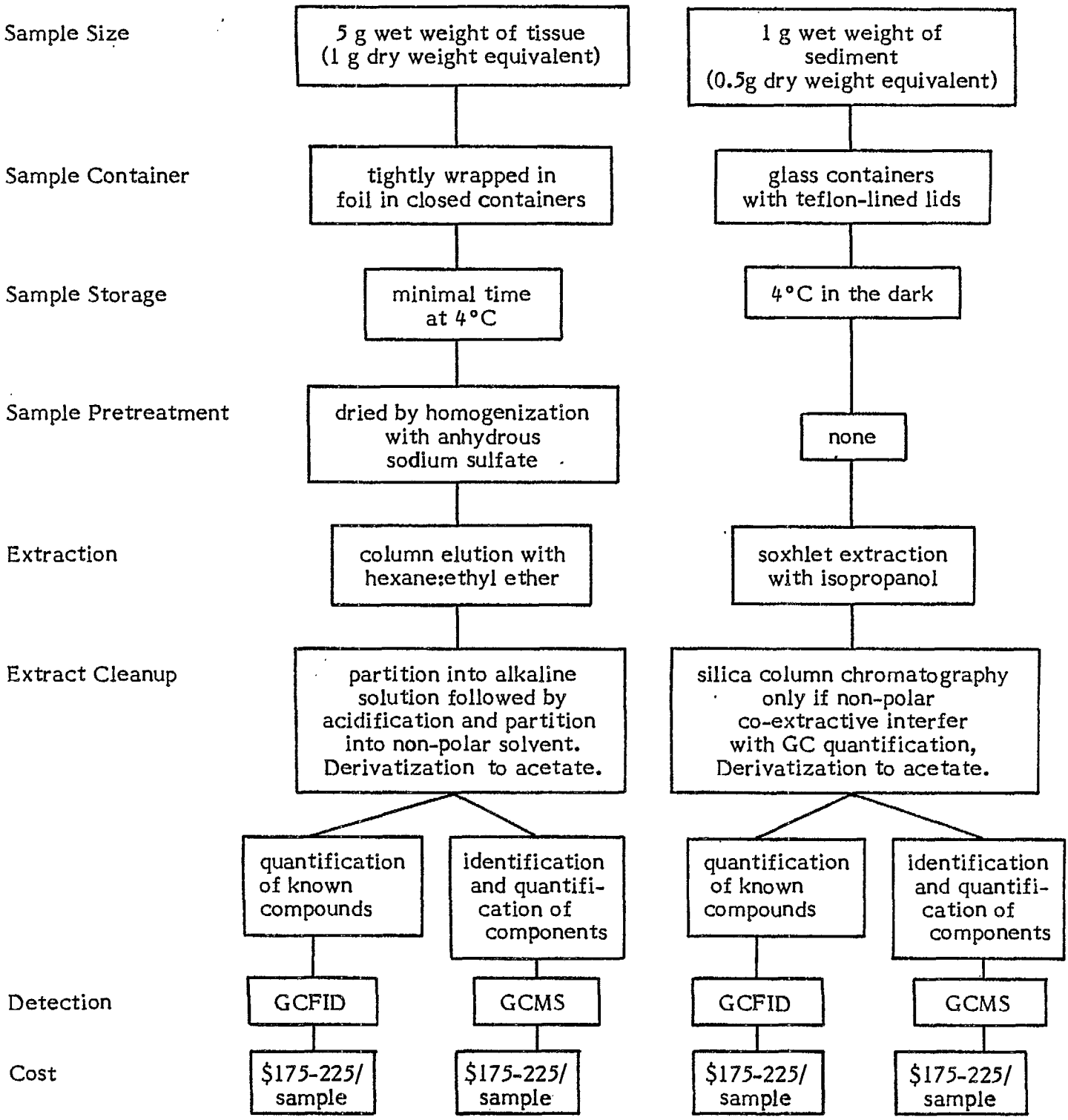
[1] The interpretation of "total phenols" by any of these methods may be difficult.

Table 7.5.3

Determination of Individual Phenolic Molecules



b) Biota

Two solvent extraction procedures are reported for specific phenol compounds in biota tissue. These methods have been applied only to a small number of phenols and it is uncertain how generally applicable they are. **The Allen and Sills (1974) method (extraction by column elution with hexane:ethyl ether) appears more rigorous than that of Hattula et al. (1978) (extraction by shaking with acidic chloroform:diethyl ether) but no convincing evidence can be given to recommend either of these methods over the other.**

Reported cleanup procedures for biota extracts include thin layer chromatography on silica gel and partitioning of the phenols into alkaline solution. The liquid-liquid partition method (Allen and Sills, 1974) is rapid and convenient and should remove co-extracted non-acidic hydrocarbon material while avoiding possible loss of phenols on the silica column.

7.5.3.2 Derivatization and Quantification Techniques

The phenol compounds in extracts are converted to less polar more volatile derivatives and determined by a gas chromatographic technique. **Formation of the acetate derivatives of the phenol molecules is recommended for this purpose (Chau and Coburn, 1974). Derivatization** procedures are discussed in detail in the section on chlorophenols (Section 7.6).

Individual phenols are determined by GC/FID or GC/MS (Coutts et al., 1980). GC/MS can detect lower concentrations and is very powerful for identifying individual components. Chlorophenol compounds can also be determined by GC/EC (see Section 7.6).

7.6 Chlorophenols

Analytical methods for chlorophenols in sediment and biota samples are reviewed in Section III-3.7 of Volume 1. Most methods are individual compound determinations: chlorophenol compounds are separated, identified and quantitated individually. Although the total concentration of chlorophenols can be estimated by summing the level of the individual components identified, this is seldom required. It is more likely that "total phenols" levels are of interest and details of this analysis are discussed in Section 7.5. Methods for determining individual chlorophenols in

sediment and biota samples are outlined in Table 7.6.1 and discussed below. The methods recommended are those expected to produce the most accurate and precise results. Coincidentally they are also the fastest and least expensive so that no advantage is gained in using other methods when less accuracy or precision is sufficient.

7.6.1 Sample Requirements

Approximately 10 g (wet weight) of sediment or 25 g (wet weight) of tissue are required for analysis. Careful sample handling is recommended to minimize loss of chlorophenols from the sample. Sediments are stored at -20°C in glass containers with screw-on teflon-lined lids. Glassware and containers are detergent washed, rinsed with acetone and heated at 325°C for 16 hours (EPS, 1979). Biota samples are wrapped in aluminum foil and stored in closed containers at -20°C. No data are available on acceptable storage times but it has been recommended that samples be analyzed as soon after collection as possible. Pretreatment of the samples is not required but air-drying or acidification is used in some methods. Acidification ensures that the chlorophenols are fully protonated and is useful prior to organic solvent extractions. **The effect of drying the sediment and biota samples before analysis is uncertain and is therefore not recommended.**

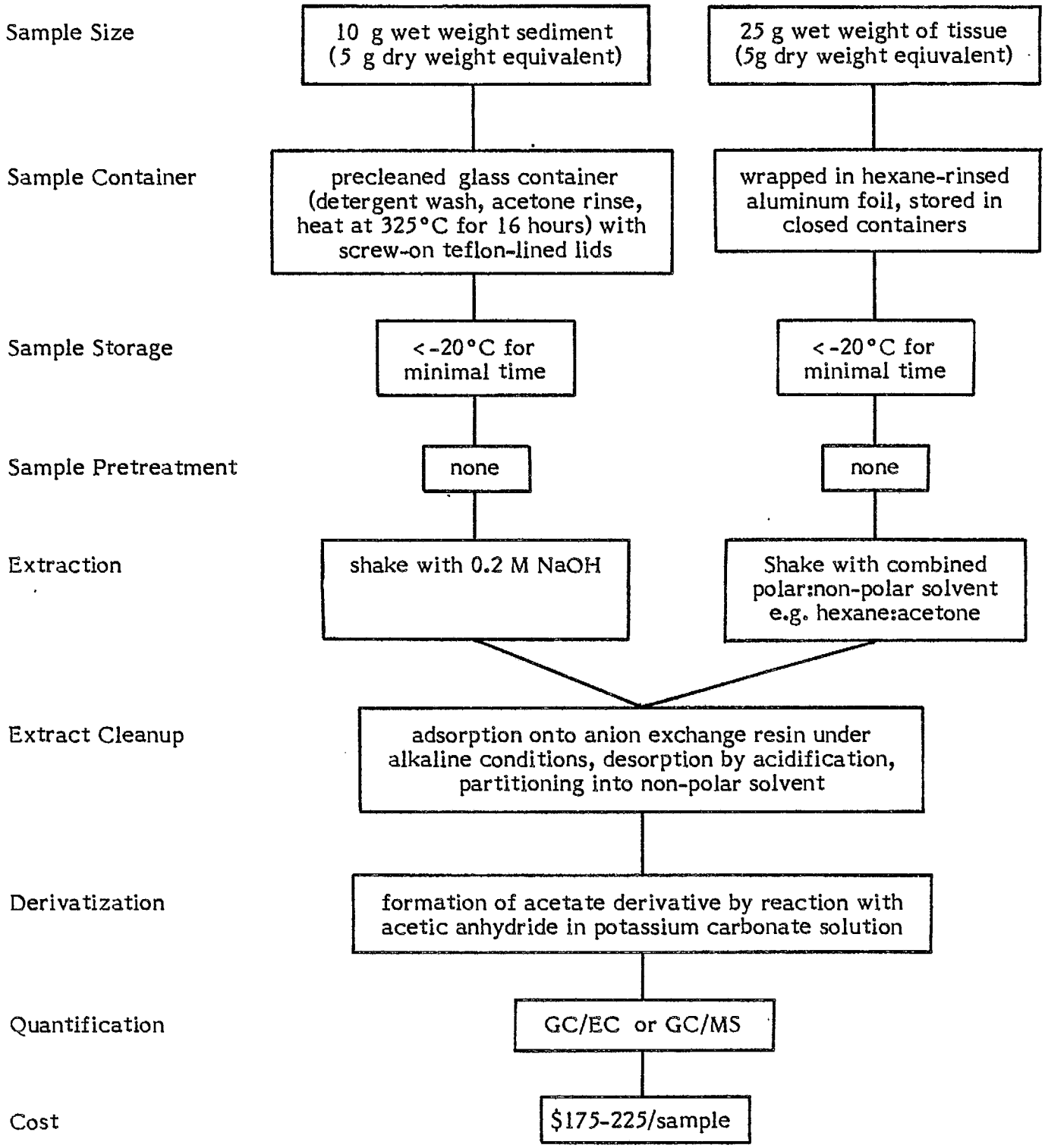
7.6.2 Extraction

a) Sediment

Several sediment extraction procedures are reported in the literature. Some of these report low or variable recoveries[1] of chlorophenols from sediments and these should not be considered for quantitative analysis. Soxhlet extraction with a mixed polar:non-polar solvent gives high recoveries with good precision. **Extraction by shaking with an aqueous sodium hydroxide solution (Renberg, 1974) also results in high recoveries of chlorophenols from sediments and has several advantages compared to soxhlet extraction.** The sodium hydroxide extraction is fast (0.5 hour compared to 24 hours), does not require large volumes of solvent, minimizes loss of volatile chlorophenols and results in cleaner extract solutions which are more readily prepared for gas chromatographic analysis.

Table 7.6.1

Determination of Chlorophenols in Sediment and Biota



b) Biota

Reported extraction techniques for tissue are predominantly organic solvent extractions. Non-polar solvents are used if the tissue is pre-dried, combined polar:non-polar solvents are used for wet tissue. The second type of extraction is recommended as pre-drying may cause loss of volatile chlorophenols. **Extraction by shaking with hexane:isopropanol or chloroform:diethyl ether or hexane:acetone followed by hexane:diethyl ether have resulted in high recoveries of chlorophenols.** Such techniques are described by Rudling (1970) and Renberg (1974) as well as others.

7.6.3 Cleanup

Two types of cleanup procedures are reported for sediment and biota extracts. The first of these is liquid-liquid extraction in which the chlorophenols are partitioned from an organic solution into aqueous alkaline solution and then partitioned into fresh organic solvent under acidic conditions. In the second cleanup

procedure the chlorophenols are adsorbed from alkaline solution onto anion exchange resin and then partitioned into fresh organic solvent by acidification (Renberg, 1974). Both procedures result in cleaned solutions suitable for gas chromatographic analysis and reasonable recovery rates of chlorophenols. Of the methods **the ion exchange cleanup is more conveniently carried out (fewer manipulations, smaller solvent volumes) and claims slightly higher recovery rates. It is therefore recommended for use on both sediment and biota extracts.**

7.6.4 Derivatization and Quantification

Chlorophenol components are converted to more volatile less polar derivatives for gas chromatographic analysis. Methyl ether, ethyl ether, trimethylsilyl and acetate derivatives have been used. The selection of a derivative is based on convenience and is not critical to the accuracy of the analysis results if correct use is made of calibration solutions and internal standards. Methyl and ethyl ether derivatives (EPS, 1979) are most often employed in the literature but the

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[1] Recovery data was reported for combined extraction-extract cleanup steps. Low reported recoveries may therefore not be due to the extraction technique, but that possibility can not be eliminated.

acetate derivatives (Chau and Coburn, 1974) eliminate interference from any organic acids still in the extract solution. They are therefore recommended for use in chlorophenol determinations. A paper by Chau and Coburn (1974) discuss their preparation in detail.

Either GC/EC or GC/MS can be used for determination of chlorophenol compounds via their volatile derivatives (Landner et al., 1977; Wegman and Van den Broek, 1983). These techniques provide similar detection limits for chlorophenols but GC/MS is more useful for identifying unknown components.

7.7 Chlorinated Hydrocarbons

Analytical methods for the determination of chlorinated hydrocarbon pesticides and PCBs in sediments and biota tissue are reviewed in Section III-3.8 of Volume 1. In these procedures pesticides and PCBs are extracted simultaneously from the samples but are separated prior to their determination by gas chromatography. Pesticides are reported as concentrations of specific individual components or as DDTs (commonly the sum of the concentrations of o,p' and p,p' - isomers of DDT, DDD and DDE). PCB concentrations are reported in terms of technical formulation equivalents, e.g. "Aroclor 1254 equivalents" or occasionally as concentrations of individual PCB compounds.

The Inland Waters Directorate (1979) and the EPA (Plumb, 1981; EPA, 1980) have published standard methods for the analysis of chlorinated hydrocarbons and PCBs in sediment and tissue samples. These methods differ in their extraction and extract cleanup steps but use similar procedures to fractionate and determine the chlorinated hydrocarbons in the extract. EPS (1979) has published a method for determining PCBs in sediment but the method is not applicable to chlorinated hydrocarbon pesticide determinations. Recommended analysis procedures for chlorinated hydrocarbons (PCBs and pesticides) in sediments and biota are outlined in Tables 7.7.1 and 7.7.2 respectively.

7.7.1 Sample Requirements

Approximately 20 g (wet weight) of sediment or 10 g (wet weight) of tissue is required for analysis. Sediment samples should be stored in glass or metal containers. The standard methods recommend either hexane extracted teflon or heavy duty

Table 7.7.1

Determination of Chlorinated Hydrocarbons in Sediments

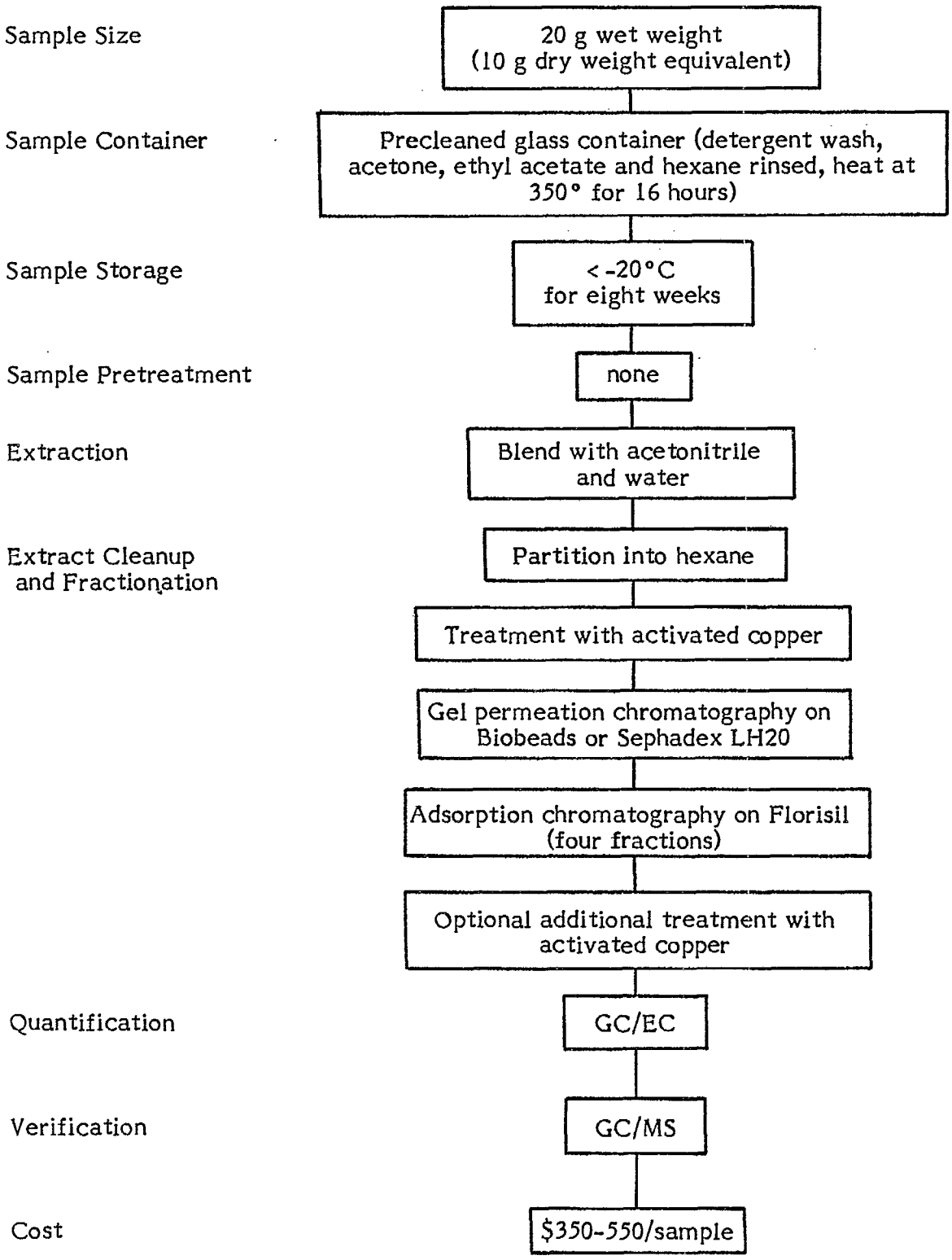
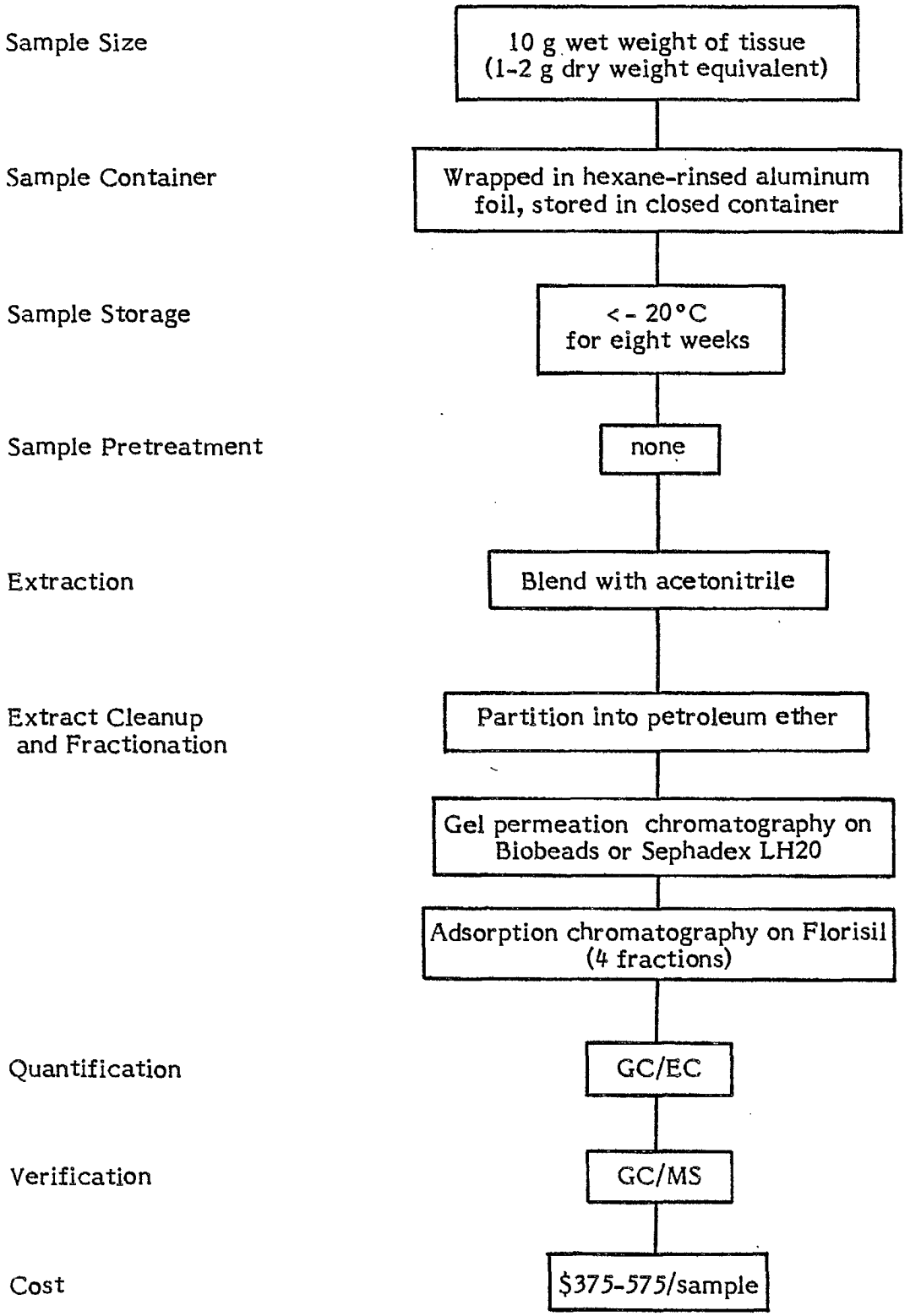


Table 7.7.2

Determination of Chlorinated Hydrocarbons in Biota



aluminum foil-lined lids for the containers (Plumb, 1981). Glassware and containers should be precleaned by washing in detergent, rinsing with acetone, pesticide-grade ethyl acetate and finally pesticide-grade hexane and heating at 350°C for 16 hours (Plumb, 1981). Biota samples should be wrapped tightly in aluminum foil and stored in closed containers. Other than teflon, **plastic containers and utensils are difficult to clean and are a common source of analytical interference (from plasticers, stabilizers, fillers, etc.), and should therefore be avoided during all stages of the analysis.** Sediment and biota samples should be stored frozen (-20°C) to minimize microbial degradation of the chlorinated hydrocarbons. Samples can be stored under these conditions for at least eight weeks but a maximum storage time has not been reported.

No pretreatment of either sediment or biota samples is required. Some literature methods call for air-drying, oven-drying, or freeze-drying of the sample before extraction but this may lead to loss of volatile chlorinated hydrocarbons. Immediately prior to analysis free water can be removed from the thawed sediments by decantation or centrifugation (Plumb, 1981; Inland Waters Directorate, 1979; Goerlitz and Law, 1974) but thawed tissue samples are analyzed without loss of fluid (Oliver and Bothen, 1982).

7.7.2 Extraction

a) Sediments

A variety of extraction techniques and solvent systems have been used for analysis of chlorinated hydrocarbons in sediments (see Table III-3.28 in Volume 1). Soxhlet extraction has been reported to result in the highest recovery of chlorinated hydrocarbons but also extracts a large amount of lipid material making extract cleanup more difficult. The EPS (1979) procedure for determination of PCBs uses a soxhlet extraction but follows it with a harsh cleanup treatment (saponification with ethanolic potassium hydroxide and oxidation with chromium trioxide) that destroys certain chlorinated hydrocarbon pesticides.

The EPA (Plumb, 1981) and Inland Waters Directorate (1979) methods extract chlorinated hydrocarbons from sediment by blending the sample with acetonitrile in the presence of water. This method provides a compromise between efficient recovery of chlorinated hydrocarbons and cleanability of the resultant extract solution. An alternate EPA procedure (Plumb, 1981) is also reported: partially dried

sediment is extracted by column elution with 1:1 hexane:acetone. No comparison of the two EPA procedures is given, nor any criteria for choosing between them, so it is assumed that they produce comparable results. The acetonitrile/blending method is more like the extraction procedures used for biota (discussed below), thus is more convenient when both types of samples are being analyzed. Other extraction procedures (e.g., sonication) could likely produce similar results but **the acetonitrile/blending method is published as a standard procedure in use in Canadian laboratories (Inland Waters Directorate, 1979) and is recommended on that basis.** No saving in time or expense is to be gained by selecting one of the alternate extraction techniques discussed in Section III-3.8.4 of Volume 1.

b) Biota

A variety of extraction techniques and solvent systems have been used for the analysis of chlorinated hydrocarbons in biota (see Section III-3.8.4.2 of Volume 1). All of these methods are reported to result in efficient recovery of chlorinated hydrocarbons from tissue samples and there is no clear reason to select one method over the others. The EPS method (1979) for PCBs in biota calls for soxhlet extraction of the tissue, but as for sediments, this results in large quantities of co-extracted material which complicate extract cleanup and fractionation. The EPS cleanup procedure, which selectively destroys certain chlorinated hydrocarbon pesticides, is not applicable to the determination of all chlorinated hydrocarbons. The Inland Waters Directorate (1979) and the EPA (1980) methods use less drastic extraction procedures. The tissue sample is blended with acetonitrile to extract the chlorinated hydrocarbons (Inland Waters Directorate, 1979) or with petroleum ether to extract the fat from the tissue; the fat is subsequently partitioned with acetonitrile to extract the chlorinated hydrocarbons (EPA, 1980). **The Inland Waters Directorate method is recommended for analysis of chlorinated hydrocarbons in biota tissue.** It is the same procedure recommended above for sediment samples and is therefore conveniently applied to biota. The EPA method was designed for use on all kinds of tissue samples, which likely explains the extra petroleum ether extraction step.

7.7.3 Extract Cleanup and Fractionation

The purpose of the extract cleanup and fractionation steps is to remove co-extracted interferences (such as lipids and elemental sulfur) from the extract and to

fractionate the chlorinated hydrocarbons into groups suitable for gas chromatographic analysis. Combinations of liquid-liquid partitioning, carbon adsorption, gel permeation chromatography and adsorption chromatography on silica, alumina and Florisil have been used.

Liquid-liquid partitioning is recommended as a rapid and effective method for the preliminary cleanup of the chlorinated hydrocarbons from co-extracted polar lipids. This procedure is particularly attractive when acetonitrile has been used as an extraction solvent as it is immiscible with saturated hydrocarbon solvents (e.g., pentane, hexane) into which the chlorinated hydrocarbons can be readily partitioned.

Extract cleanup by partitioning the chlorinated hydrocarbons from the acetonitrile extract solution into petroleum ether is described in the Inland Waters Directorate (1979) standard method and is recommended for use. Additional cleanup procedures (adsorption chromatography, gel permeation chromatography, and carbon adsorption) are desirable for heavily contaminated sediment extracts or biota. As adsorption chromatography on silica or alumina may result in some loss of chlorinated hydrocarbon components, **gel permeation chromatography on Biobeads SX-3 or Sephadex LH20 is recommended for additional cleanup.** It is the least reactive cleanup material, gives high recoveries and can be automated. Such a procedure has been described by the EPA (1980) for the cleanup of biota extracts for chlorinated hydrocarbon analysis.

Adsorption chromatography on Florisil columns is the recommended method of separating chlorinated hydrocarbons into groups suitable for GC analysis. This method is well characterized in the literature and is used in all the standard methods. It is described in detail by Plumb (1981) and in the Inland Waters Directorate method (1979). Four fractions are obtained from the Florisil column and each is analyzed separately. A listing of chlorinated hydrocarbons found in each fraction is given by Plumb (1981) and in the Inland Waters Directorate method (1979). If PCBs and/or a complex mixture of chlorinated hydrocarbon pesticides are present in the sample, the extract must be fractionated on the Florisil column to ensure that chromatographic peaks due to the individual components can be resolved.

Elemental sulfur must be removed from sediment extracts prior to quantification by gas chromatography (discussed below). Treatment of the extract with either mercury or copper has been shown to effectively remove the sulfur interference with no effect on chlorinated hydrocarbons in the solution. **Treatment with activated copper (Plumb, 1981) is the recommended method** as it is less hazardous and generally requires only one application (repetitions of the mercury treatment may be required).

7.7.4 Quantification

Gas chromatography with either electron capture (GC/EC) or mass spectral detection with selected ion monitoring (GC/MS/SIM) is used for quantification of chlorinated hydrocarbon pesticides and PCBs (Ernst et al., 1976; Castelli et al., 1983; Plumb, 1981; EPS, 1979). These techniques are extremely sensitive for chlorinated compounds and detection limits of approximately 0.1 $ng \cdot g^{-1}$ can be obtained for both sediment and tissue samples. Both GC/MS and GC/EC provide rapid, reliable quantification of chlorinated hydrocarbons and the choice of technique should be based upon cost, availability and the selectivity required. GC/EC is more common and less expensive. GC/MS, although less commonly used, is more selective and can, at a lower sensitivity, provide full mass spectral confirmation.

a) Chlorinated Hydrocarbon Pesticides

GC/EC and GC/MS/SIM use similar approaches of calibrated instrument response to infer and quantify a target compound. As in any GC method, a single chlorinated hydrocarbon assignment in a GC/EC chromatogram is based upon the detection of a peak within the narrow retention time window observed for the authentic material. An assignment is only considered as an inference if that compound is expected to be present in the appropriate Florisil column fraction. With GC/MS/SIM, additional confirmation is available as several characteristic ions of the analyte may be monitored and only when these ions co-vary and maximize at the appropriate retention time with the same relative ion abundance as the standard, is that compound confirmed.

b) PCBs

The identification of PCBs by GC/EC or GC/MS uses the same criteria as for the single compound GC analyses (Section 7.3.4a) but is complicated by the complex nature of these products. For analyses using GC/EC, the appearance in a chromatogram of the appropriate Florisil fraction (fraction 1) of peaks within the retention time windows as found in an authentic PCB calibration standard and

exhibiting a similar distribution of GC peak intensities, is taken as an inference of that PCB formulation. The alteration of PCB composition in the environment by multiple sources, physical fractionation, degradation and photolysis will result in an environmental PCB extract that will not correspond to any particular commercial PCB formulation. Several approaches have been used to resolve this problem, the most common being quantification as a commonly encountered PCB formulation, quantification as individual components or by perchlorination of the PCB to decachlorobiphenyl and its analysis as a single compound. Analysis of individual components is difficult by GC/EC and the perchlorination technique does not provide compositional information and can result in the detection of PCBs from the chlorination of artifacts. The recommended technique by GC/EC is the quantification of the PCB pattern as a commonly encountered PCB formulation, usually in North America, Aroclor 1254 or Aroclor 1260. The response is measured as the sum of several (i.e., three or more) prominent GC peaks and this is related to the response to a known quantity of internal standard.

GC/MS/SIM uses a similar approach with a series of characteristic ion chromatograms. The appearance in the appropriate florisil fraction of peaks within the determined retention time windows is taken as confirmation of the PCB and quantification is based on the PCB/internal standard response taken.

The quantification of individual PCB compounds is possible by high resolution GC/MS/SIM and this gives the greatest accuracy as no compositional approximations are necessary. However, such data is difficult to compare to existing data based upon whole PCB analyses and this approach, while attractive, has not been extensively used.

8. MEASUREMENT OF SEDIMENT GRAIN SIZE

8.1 Introduction

Grain size is an important and fundamental sediment property that has application in a number of areas. Grain size distributions offer information on despositional environments, sedimentation processes and on the geotechnical properties of the bulk sediment. In environmental studies, sediment grain size is important in comparing and evaluating chemical composition and benthic biota. Many environmental pollutants are scavanged from the water column through sorption processes with settling particulates. The effective surface area of particulates increases in inverse proportion to particle size so that the finer particulates have a much larger effective surface area for a given weight of material. The finer size particulates also include phases with a higher trace metal content than those of the coarser material. As a result, most trace metals and organics are partitioned into the fine size range (silt and clay) and are not evenly distributed within the sediment matrix. A comparison of the chemical composition of sediments and associated benthic communities therefore must be made on the basis of grain size characteristics.

It is usual to describe sediments on the basis of their gross grain size characteristics as gravel, sand, silt, clay or combinations thereof. These terms correspond to a broad range of particle sizes (generally taken as mean particle diameter) ranging from less than a micron to several centimetres. In North America, the Wentworth classification system is most widely used to describe grain size characteristics. The definition of sediment types in terms of particle diameter using the Wentworth classification is shown in Table 8.1. Actual methods for grain size analysis measure quite different properties of individual particles which relate in various ways to actual size. Sieving techniques are the most popular method and discriminate sediment particles on the basis of their smallest cross-sectional diameter. Settling tube or sedimentation methods measure the settling velocity of particles in water. Electronic particle counting methods determine particle size on a volume basis. Microscopic analysis of fines gives measurements which are most directly related to actual grain size as well as information on grain shape.

TABLE 8.1

RELATIONSHIP BETWEEN VARIOUS SIZE UNITS, U.S. STANDARD SIEVE SIZES AND WENTWORTH CLASSIFICATION

	U.S. STANDARD SIEVE MESH	MILLIMETERS	PHI (ϕ) UNITS	WENTWORTH SIZE CLASS	COMMON TEST PROCEDURES
GRAVEL	5	16	-4	Pebble	Sieve analysis
		4	-2		
	6	3.36	-1.75		
	7	2.83	-1.50		
	8	2.38	-1.25	Granule	
	10	2.00	-1.0		
SAND	12	1.68	-0.75		
	14	1.41	-0.5	Very Coarse Sand	
	16	1.19	-0.25		
	18	1.00	0.0		
	20	0.84	0.25		
	25	0.71	0.5	Coarse Sand	Settling Tube or Sieve
	30	0.59	0.75		
	35	0.50	1.0		
	40	0.42	1.25		
	45	0.35	1.5	Medium Sand	
	50	0.30	1.75		
	60	0.25	2.0		
	70	0.210	2.25		
	80	0.177	2.5	Fine Sand	
	100	0.149	2.75		
	120	0.125	3.0		
	140	0.105	3.25		
	170	0.088	3.5	Very Fine Sand	
	200	0.074	3.75		
	230	0.0625	4.0		
SILT	270	0.053	4.25	Very Coarse Silt	Sedimentation techniques: Pipette, Hydrometer; Optical Sensors X-ray
	325	0.044	4.5		
	-	0.037	4.75	Coarse Silt	
	-	0.031	5.0		
	-	0.0156	6.0	Medium Silt	
	-	0.0078	7.0	Fine Silt	
	-	0.0039	8.0	Very Fine Silt	
CLAY		0.0020	9.0		
	-	0.00098	10.0	Clay	
	-	0.00049	11.0		Practical Limit of Sedimentation Analysis
	-	0.00024	12.0		
	-	0.00012	13.0		
	-	0.00006	14.0		

$\phi = -\log_2$ diameter (millimeters)

No one method is capable of measuring grain size over the full range of gravel to clay size particles. For a detailed analysis of sediments with broad range of particle size, at least two methods will be required. Traditionally, sieving methods have been used to separate particles in the sand and gravel size categories (down to about 63 μm) while pipet and hydrometer sedimentation techniques have been used for size measurements of the clay and silt fractions (0.5 μm to 63 μm). Sedimentation techniques can also be used for sand analysis (particles to 2 mm) and numerous automated methods have been developed using this principle.

Electronic particle counting techniques have a wide theoretical operating range. These methods have become fairly standardized because of the available instrumentation and are a recommended method of the EPA (Plumb, 1981). Sieve, sedimentation and electronic counting techniques are the methods considered in the following. Microscopic procedures have not been included because of their limited use and expensive instrumentation requirements. Further information on these techniques can be found in Swift et. al, (1972).

8.2 Selection of a Suitable Method

The main factors to be considered in selecting a method for grain size analyses are:

- **detail required and type of sediment**
- **number of samples to be analyzed**
- **amount of material available**
- **availability of previous data and type of method used**
- **cost**

Grain size analyses in environmental sampling are usually carried out as supporting information. Samplers are chosen to meet the requirements of other parameters (benthos, trace metals or hydrocarbons), not grain size. The method of grain size analysis must usually fit the available sample size. Number of samples and cost are related. If a very large number of samples are to be analyzed, then automated methods have definite advantages in terms of time per analysis and hence cost. If analyses are to be done in a commercial laboratory, usually only classical sieve or pipet analyses can be done. Sieve and pipet or hydrometer analysis will also

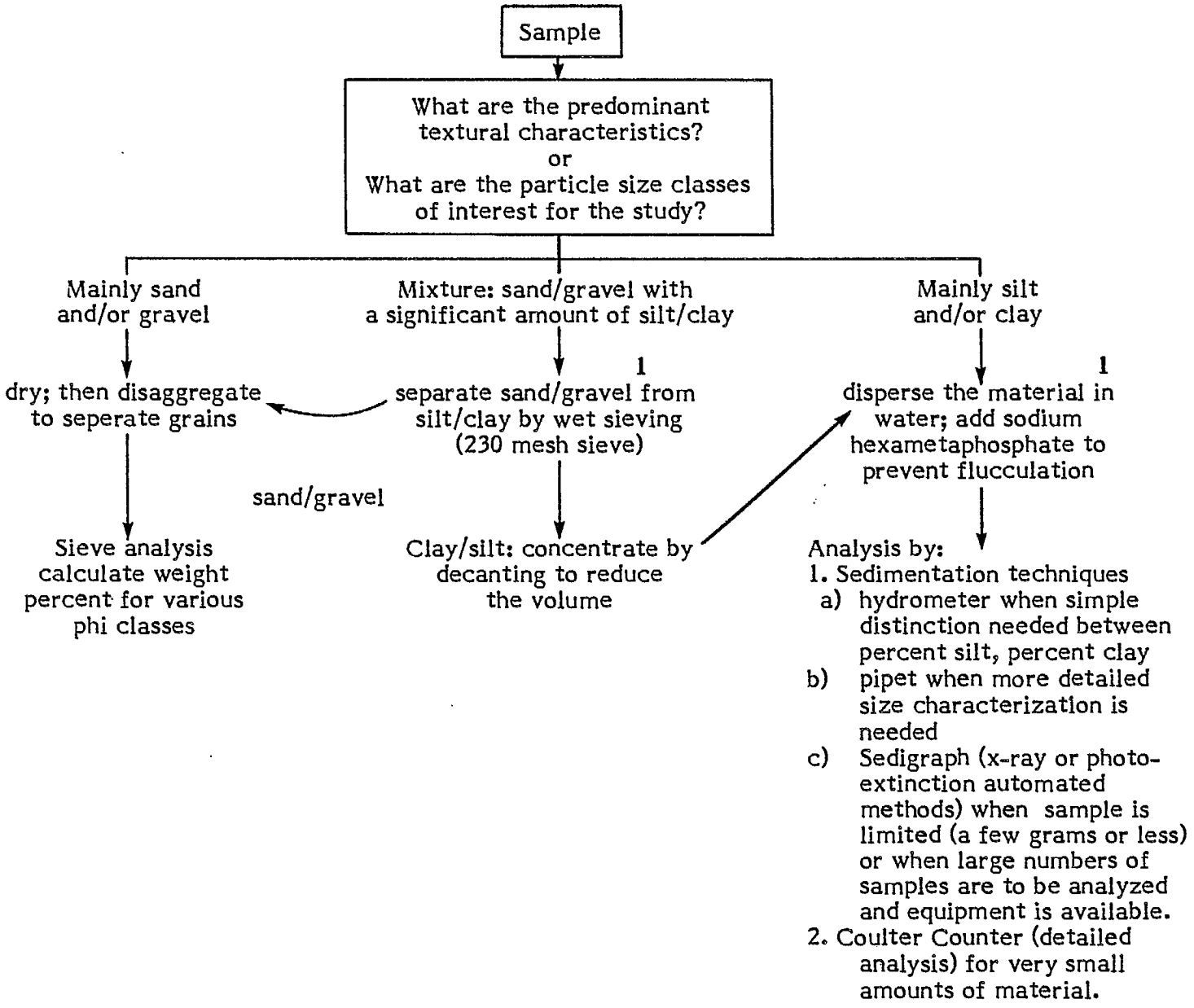
be the methods used in almost all historical data. Figure 8.1 provides a guide to method selection on the basis of particle size and size of sample. Advantages and disadvantages of the various methods and background information is given in Volume 1, Section III-4. A summary of performance characteristics, sample requirements and relative cost for the most common methods (sieve, pipet and hydrometer) is given in Table 8.2.

8.3 Sample preparation for Grain Size Analysis

Samples for grain size analysis are generally stored cool or frozen in a sealed container to prevent moisture loss. Sample size will vary with the type of sediment and analysis technique. Course sediments will require from 50 - 100 g or more for sieve analysis, while 20 g or less will suffice for fines by pipet analysis.

Sample preparation methods are somewhat arbitrary and depend on the final use of the data, properties of the sediment and the precision required. A sedimentologist, for instance, would wish to break-up any particulate aggregates while the environmentalist may wish to know environmental particle size and therefore aggregate size. In all cases however, the goal of sample preparation is to ensure that the measurements made of size distribution reflect natural variations and are not artefacts of sample preparation (Nelsen, 1983). Nelsen emphasized that a consistent manner of sample preparation is crucial to the final interpretation of data. Depending on the sediment type and the degree of splitting or dilution required, sample preparation times may equal or greatly exceed analysis time. This is especially true in automated methods.

Walton (1978) outlined a generalized scheme that is widely used for the preparation of samples for analysis by sieve and pipette techniques (Section III - 4, Volume 1). Because of its widespread use, this method is recommended. Samples are usually wet-sieved initially through a coarse 16 mm mesh sieve to remove cobbles. The sediment passing the sieve can be treated in a number of ways before further analysis and depending on the techniques to be used. The goal of these procedures is to prevent particles from aggregating or sticking to equipment. Organic material if present in large quantities (1-2% or more) is usually removed, generally by boiling with H_2O_2 (exact procedures can be found in Walton, 1978 or Carver, 1971). Depending on the method to be used, the remaining sediment is then wet sieved to separate the "fines" from gravel and sand for sieve and pipet analysis or just to separate gravel for settling tube or electronic particle counting methods. If analysis



Note:

1) Preparation times for a mixed sediment or for techniques requiring a small amount of material will be much longer than the analysis time.

Figure 8.1 Choice of Grain Size Analysis Procedure

TABLE 8.2

SAMPLE REQUIREMENTS AND PERFORMANCE CHARACTERISTICS OF SIEVE AND PIPET AND HYDROMETER SEDIMENTATION TECHNIQUES

	PIPET	HYDROMETER	SIEVE
Sample Requirements	5 - 25 g	50 - 100 g	50 g sand 100 g gravel
Practical Range of Application	0.5 - 63 μm	∿1 - 63 μm	∿50-16,000
Time Required	almost 3 days required to carry out analyses to 0.5 μm; 17 hours to 1 μm.	slower than pipet analysis for equivalent particle size (24 hours to 0.1 μm)	∿4 hours for detailed analysis
Comments	more precise than hydrometer because of less disturbance of water column. precision (CV) of approx. 0.5 ∅ attainable	less precise but less exacting procedure than pipet. Suitable for a field lab as no drying or weighing required	most economical and common method for sand analysis: only practical means of gravel analysis
Cost	$60-120/sample depending on detail	$40-60/sample	$35-50/sample
References	Walton, 1978; Krumbein and Pettijohn, 1938; Carver, 1971 Folk, 1974	Head, 1981; Krumbein and Pettijohn, 1938	Walton, 1978

of silt and clay size particles is required, the fines passing a 230 mesh sieve must be concentrated, either by centrifuging or allowing time for settling and then decanting excess water. Either method is time consuming especially where a large volume of water has been used in wet sieving.

For sieving, or settling tube methods the sand and gravel fraction is usually dried, weighed then disaggregated before analysis. Disaggregation is usually not a problem with clean sand and gravel.

The fine fraction if dried in an oven tends to be cohesive especially if there is a lot of clay present and the method of disaggregation critical to the final result. Freeze drying is preferable to oven drying for samples with a high clay content. Low temperature ashing is also an effective means of sample preparation (Kranck and Milligan, 1979). Nelsen (1983) compared four methods for disaggregation namely 1) soaking with Calgon, 2) shaking in water with a small amount of Calgon 3) ultrasonification and 4) stirring with a malt-mixer type stirring device. On the basis of the tests, Nelsen reached the following conclusions:

1. Under similar conditions of disaggregation and analysis there is no statistically significant difference between the size distributions of freeze or air-dried portions of a given sediment.

2. The disaggregation time needed to arrive at the terminal distributions seem to partition the methods into two groups, the very rapid (stir and ultrasonify) and the variable (shake and soak) disaggregation times.

3. From the data available the methods with variable disaggregation times seem to be sample dependent.

4. For a given sample the terminal distributions produced by all the disaggregation methods were statistically indistinguishable at the 95% confidence level.

The addition of a dispersant is usually required. The most commonly used dispersant is sodium hexametaphospate (Calgon) which is added to a suspension of the fines in sufficient quantity to inhibit aggregation and accelerated settling velocities.

Techniques using very small samples such as electronic counting and automated settling tube methods require more care in sample preparation as the population size is much smaller. This will involve microsplitting of samples to obtain representative subsamples which is more time consuming than subsampling for much larger weights.

8.4 Data Presentation

There are numerous means of presenting grain size data depending on the intended use and detail required. **As a minimum for environmental studies, the following should be provided:**

(1) tables of actual weights used and the weights in various size fractions.

(2) for settling tube methods, data on timing and methods of calculation (assumptions used).

Graphical presentations of data should either be in the form of a cumulative frequency distribution curve or a triangular plot (see Volume 1, Section III - 4) as these have widespread use and are useful for comparing a large number of samples at one time.

9. LEAD-210 AS A MEASURE OF SEDIMENT ACCUMULATION RATES

9.1 Background

The rate at which sediment accumulates is an important consideration in evaluating depth related features in sediment cores and in predicting the impact of anthropogenic inputs relative to natural fluxes. There are a number of methods for estimating rates of sediment accumulation depending on location and sediment characteristics. Direct measurements of the downward particle flux can be made by trapping settling water column particulates. Estimates can also be derived from stratigraphic inferences based on total depth of sediment in relation to the estimated time of deposition or from budget calculations relating to the particulate flux associated with river run-off and the concentration of particulates in the water column. Finally analysis of sediment cores can be made based on paleontology and radiometric methods. In general, no single method is universally applicable and it is desireable to obtain estimates from two or more methods to improve reliability. The determination of geochronologies using artificial and natural radionuclides has become well established as a means for dating marine deposits over periods of time of tens to millions of years (Turekian and Cochran, 1978; Goldberg and Bruland, 1974). From an environmental perspective, recent processes are most important and this reduces the number of useful radionuclides considerably.

Lead-210 is the most commonly used natural radionuclide for dating recent sediments. Its chemistry and half-life permits dating for periods of 100-150 years. Pb-210 methods have been reviewed in detail by Robbins (1978) and have been applied to a variety of lake and coastal marine deposits. Dating using a natural radionuclide is based on the decay of the isotope after incorporation into the sediment.

Lead-210 is a member of the U-238 natural radioactive series and has a half-life of 22.3 years. There does not appear to be a significant amount of Pb-210 produced in nuclear explosions.

Atmospheric fluxes of Pb-210 in arctic regions are lower than in more temperate regions. Naidu et al., (1981) have reported an average flux of 0.08 Bq cm^{-2} y^{-1} for the Alaskan Beaufort Shelf. This is an order of magnitude lower than fluxes measured in the northeastern United States and Californla.

For practical purposes, the method provides estimates of time scales of up to 5 half-lives (ℵ 100-110 years). In ideal conditions, (i.e. undisturbed sediments) the unsupported or excess Pb-210 concentrations exhibit an exponential decay in activity with depth of burial. However, sediments are often disturbed by biological or physical processess and by variation in sedimentation rate which purturbs the expected exponential decay curve. This can lead to problems in data interpretation or erroneous results. **For this reason, it is important that a sedimentation rate estimate be obtained from an alternate method for comparative purposes.**

9.2 Sampling for Pb-210

Sampling for Pb-210 dating places added emphasis on collecting the sediment surface intact. The factors to be considered when sampling and subsampling are summarized in Table 9.1.

9.3 Choice of Analytical Method

There have been two basic approaches to Pb-210 measurements in sediments. Neither involves the direct measurement of Pb-210 since it is a low energy β emitter (Emax = 61 kev). The most common approach involves the extraction and purification of Pb from the sediment via an acid leach and precipitation and counting of the β decay of Bi-210 (Emax = 1.17 MeV), the daughter product of Pb-210 decay. The other approach used involves extracting and isolating Po and Pb from the sediment and counting the α decay of Po-210 (Emax = 5.3 MeV) which is assumed to be in secular equilibrium with Pb-210. Determination of either Pb-210 or Po-210 is a multi-step process. Briefly, the metal is extracted from the sediment by a hot acid leach, purified by extraction from the leachate and then collected as a precipitate. Po-210 is measured directly, while Bi-210 is allowed to equilibriate with Pb-210. Recommended methods are summarized in Table 9.2. Two choices are given: 1) collection and direct measurement of Po-210, and 2) collection of Pb-210 and subsequent measurement of Bi-210 after a suitable equilibration time. **It should be stressed that whatever method is used, a suitable carrier or tracers (see Table 9.2) be used to determine the yield.** As the use of the method lies in relating changes in Pb-210 concentrations, it is imperative that variations in yield be taken into account.

TABLE 9.1

FACTORS/RECOMMENDED PROCEDURES FOR Pb-210 SAMPLING, SUBSAMPLING AND STORAGE

STEP	RECOMMENDED PROCEDURE	COMMENTS/REFERENCES
Type of Sediment	only sediments with a high proportion of silt and clay should be considered for dating	Pb-210 levels in Arctic sediments are generally low. Pb-210 as for other trace elements, is concentrated in the silt/clay fraction
Length of Core Required	20 - 50 cm	length must be sufficient so that excess Pb-210 will have decayed away. This will take 100-150 years. Sedimentation rates will rarely exceed 1 mm/y.
Type of Sampler	1. Box corer or damped cylindrical gravity corer 2. cylindrical gravity corer with slowed entry.	Section 3 (Choice of Samplers) It is crucial that the core be undisturbed and that surface "flocs" and fines be recovered.
Handling of Core in Field	1. keep upright 2. carefully draw off surface water with a siphon 3. skim off surface floc if present and store separately	retain some surface water for later salinity analysis (necessary for porosity calculation) plastic bottle/bag; and freeze
Storage	4. extrude core and section or freeze the entire core in the core barrel or liner after surface floc is removed. Core sections stored in plastic bags and frozen.	
Extruding Cores	A. IN FIELD if convenient, cores can be extruded with a tight fitting piston device B. IN LAB Frozen cores can be thawed slightly and then extruded and cut using a band saw or thawed completely and handled as in the field.	Kemp et al., 1971 Before sectioning, material next to the sampler wall should be removed as it may be contaminated
Sectioning	thin sections (0.5 - 1.0 cm) are required for the upper 10 cm. Thicker sections can be taken at greater depths. Extruded, frozen cores may be cut with a band saw using carbide tipped blade. Thawed or unfrozen core sections should be cut with a knife.	take as many sections as possible. All sections need not be analyzed
Sample Requirements	Each section should yield at least 1-5 g dry sediment.	

TABLE 9.2

RECOMMENDED ANALYTICAL METHODS FOR Pb-210 DATING

METHOD	REFERENCE	QUANTITY OF SEDIMENT REQUIRED	DIGESTION	SEPARATION AND PURIFICATION OF Pb	RECOVERY/PRECISION	COUNTING	COST
1. Pb-210 via Bi-210	Koide et al., 1972 Krishnaswamy et al., 1971; Rama et al., 1961	2 - 10 g	initial heating at 400°C for 2-3 hours to remove organics. Residue leached in 6N HCl for 1 hour at 100°C. Leachate dried and dissolved in 1.5N HCl	30 mg Pb^{2+} (Pb-210 free $PbNO_3$ solution) added as carrier: solution passed through anion exchange column: Pb elected with 1.5N HCl: Pb is precipitated at Pb 504 from pH 2 solution	not stated	made after allowing 20-40 days for in growth of Bi-210 daughter in flow-type beta counter.	$50 - $75/ sample
	Matsumoto and Wong, 1977. Macdonald et al., 1983	~3 g	20 mL conc. HNO_3 at 120°C for 2 hours: leachate diluted to 3N HNO_3	20 mg Pb^{2+} (Pb-210 free $PbNO_3$ solution) added as carrier: Pb separated by electrodeposition on a Pt anode as PbO_2. PbO_2 dissolved in 3N HNO_3 containing a few drops of H_2O_2 Pb precipitated as Pb S04 from pH 2 solution	70-95% recovery of Pb	made after allowing 30 days for in growth of Bi-210 daughter in flow-type beta counter	~$100/sample
2. Pb-210 via Po-210	Robbins and Edgington, 1975 Cliffton and Hamilton, 1979 Flynn, 1968	up to 5 g	HNO_3 followed by several aliquots of conc. HCl. HCl treatment continued until no NO_2 fumes evolved. Solution diluted.	- measurement of Po-210 - assumed to be in secular equilibrium with Pb-210. Filtrate is evaporated to small volume and heated with conc. HCl to eliminate any remaining HNO_3. Po-210 self plated onto silver planchettes with 10 mg of a bismuth carrier at 85-90°C in the presence of sodium citrate at pH 2. Plating times 60 min for 79% recovery.	quantitative recovery of Po-210	Po-210 alpha-activity counting done for 10^3 min. (2)	$75-$100/sample
	Nriagu et al., 1979	1 - 3 g	15 mL aqua regia/g sediment heated at 90°C for 6 hours with stirring	plating times 4 hours with shaking in presence of ascorbic acid (1 g/3 g sediment)		NMC 2 proportional counter	
	Farmer, 1978	1 g	100 mL 8M HNO_3 for 4 hours at 70°C	Pb-208 added during digestion to monitor yield			

9.4 Data Interpretation

There have been three basic models developed for interpreting Pb-210 depth profiles and estimating accumulation rates (Appleby and Oldfield, 1983); 1) constant flux, constant sedimentation rate (CF:CS) model; 2) constant rate of supply (CRS) model and 3) constant initial concentration (CIC) model. The models and their application and limitations are described in Volume 1 and references therein.

No single model is applicable in all cases, while in some instances no model may be suitable. Appleby and Oldfield (1983) have outlined a tentative procedure for choosing which model to apply:

1. Linear profiles (ln excess Pb-210 activity vs depth): all models give the same chronology

2. Non-linear profiles

   a) if the total cumulative excess Pb-210 (residuals) in a core is comparable with known atmospheric flux or with levels in nearby cores, then the C.R.S. model is likely applicable.

   b) if Pb-210 residuals vary from different sites and are in proportion to sediment accumulation rates, then the CIC model is most likely applicable.

   c) if neither case (a or b) is satisfied, dating cannot be reliably carried out.

Although the CRS and CIC models make one less assumption than the CF:CS model, they require more detailed sampling intervals and are subject to false interpretation. Physical and infaunal mixing as well as episodic events such as slumping or ice scouring would result in non-linear (ln A vs z') plots and could be interpreted as changes in sedimentation rate or flux. For these reasons, the dating of sediment cores must be done with caution and supporting data sought from independent dating methods and other measurements which might indicate whether or not the sediment has been mixed or disturbed. Swan et al, (1982) have noted that supported Pb-210 may not be constant with depth and that Ra-226 measurements should be made to compensate for this.

10. QUALITY ASSURANCE IN ANALYTICAL METHODS

Quality assurance is a term used to describe a program designed to reduce measurement errors and to assure that the results have a high probability of being of acceptable quality. The goal of a quality assurance program is to bring a measurement system into a state of statistical control and subsequently maintain it there. Statistical control implies that procedural errors have been reduced to acceptable levels and have been characterized statistically. A quality assurance program embodies two concepts: **quality control,** the mechanism established to control errors; and **quality assessment,** the system used to verify that control is working (Taylor, 1981). The topic of quality assurance has been discussed in detail in several recent reviews (Taylor, 1981; Keith et al., 1983; Kirchmer, 1983)

Quality has a subjective definition. Acceptable data in one study may be found lacking in another. There are no set rules that one can apply. It is only possible to outline some of the elements that go into quality control and assessment. The actual methods used will be dependent on the intended use of the data, the laboratory and demands of the situation. **However, some type of quality assurance program must be incorporated into all studies so that quality statements can be made.** If no information is available on the data which will allow statements of precision and accuracy, then the data is of little value.

A guide has been recently produced for obtaining, calculating and reporting quality statements for chemical analysis of marine sediments.[1] The guide includes discussions of general concepts, terminology, systematic and random errors, and provides detailed recommendations for quality control and assessment. Several worked examples are provided to illustrate the appplication to actual data sets. **This guide should be consulted in designing a quality assurance program.**

An important first step in the use of any analytical procedure is validation of the method. This means establishing key performance characteristics (accuracy, precision, detection limit, blank) and bringing the method into a state of statistical control (Taylor, 1983). Some specific quality control and assessment procedures that should be used in ongoing marine sediment and biota analyses are:

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1 Macdonald, R.W. Guidelines for Obtaining, Calculating and Reporting Quality Statements for Chemical Analysis of Marine Sediments. A copy of this report may be obtained by contacting Dr. R. Macdonald, Institute of Ocean Sciences, Department of Fisheries and Oceans, Sidney B.C. (604)-656-8409.

a) analysis of reference sediments and tissue,

b) analyses of blind replicates and control samples,

c) use of control charts for assessing the quality of the results obtained for items a and b, as well as blanks and standard solutions and

d) collaborative testing programs.

For many organic compounds, reference materials are not available and quality assurance programs must emphasize components of the measurement system such as blanks, replicate analyses, spike recoveries and collaborative testing. It has been suggested that the cost of quality assurance should be 10-20% of the total analytical costs:

10.1 Certified Reference Materials

10.1.1 Metals

A list of reference materials (sediments and tissue) that have certified levels for key metals is given in Table 10.1. A list of suppliers is given in Table 10.3.

10.1.2 Organics

A list of reference materials and intercalibration materials (i.e., no certified values available) is given in Table 10.2 for organics. No certified reference materials are available at present for biota although some are under deveopment (Russell, 1984). Other reference materials that may be useful for instrumental calibration are also listed.

10.2 Documentation and Reporting

The requirements for documentation and data reporting are outlined in Macdonald's Guide. The key requirements are summarized below:

Table 10.1

Standard Reference Maerials for Metals in Marine Analytical Chemistry
(from Russell, 1984)

	Name	Metals (certified values are available)	Supplier
Sediments	Estuarine Sediment, SRM 1046	As, Cd, Co, Cu, Pb, Mn, Mg, Ni, V, Zn, Al, Ca, Fe, Mg, and P	NBS
	Marine Sediments, MESS-1	Be, V, Cr, Mn, Co, Ni, Zn, As, Sb, Cd, Hg, Pb, C, Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti and Fe	NRCC
	Marine Sediments, BCSS-1	Be, V, Cr, Mn, Co, Ni, Zn, As, Sb, Cd, Hg, Pb, C, Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti and Fe	NRCC
	Marine Mud, MAG-1	Al_2O_3, Fe_2O_3, B*, Ba, Be*, Cd*, Co, Cr, Cs*, Cu, Ga*, Hf*, La, Li*, Mn, Mo*, Nb*, Ni, Pb*, Rb, Sb*, Sc, Sn*, Sr, Ta*, Th*, U*, V, Y, Zn, Zr	USGS
Biological Materials	Oyster Tissue, SRM 1566	As, Cd, Cr, Cu, Fe, Pb, Mn, Hg, Ni, Rb, Se, Ag, Sr, U, Zn, Ca, Mg, K, Na	NBS
	Lobster Hepatopancreas, TORT-1	V, Cr, Mn, Fe, Co, Ni, Cu, As, Se, Sr, Mo, Cd, Hg, pb, Na, Mg, P, S, Cl, K and Ca	NRCC
	Chlorella, NIES No. 3	K, Ca, Mg, Fe, Mn, Sr, Zn, Cu, Co	NIES
	Mussel Powder, NIES No. 6*	Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sr, Ti, Zn, Ca, K, Mg, Na, P	NIES
	Albacore Tuna, RM 50*	Se, Zn, As, Hg, Mn, Na, K, Pb	NBS

* Research material, values not certified.

Table 10.2

Available Certified Reference Materials and Intercalibration Samples for Organics

a) Certified Reference Materials

a.1 Sediments

I.D.	Source Agency	Certified Components	Comments
CS-1	NRC Canada	PCB's (Aroclor 1254)	Coastal marine sediments dried, homogenised
HS-1	NRC Canada	PCB's (Aroclor 1254) PCB's 10 individual isomers	Harbour sediments
HS-2	NRC Canada	PCB's, Aroclor 1254 PCB as 10 individual isomers	Harbour sediment
EC-1	CCIW	Organochlorine compounds PAH, PCBs chlorobenzene	In preparation Lake sediment
EC-2	CCIW	Organochlorine compounds PAH, PCBs chlorobenzenes	In preparation Lake sediments

a.2 Biota

None available

b) Intercalibration Reference Materials

b.1 Sediments

I.D.	Source Agency	Certified Components	Comments
Duramish II	NOAA/BLM	alkanes PAH isoprenoids	River sediment

b.2 Biota

I.D.	Source Agency	Certified Components	Comments
MA-A-1	IAEA	5 chlorinated pesticides 2 PCB's (Aroclor 1254)	Cocepod Homogenate 15 labs participated
MA-M-2	IAEA	7 chlorinated pesticides 2 PCBs (Aroclor 1254,1260)	Fish flesh homogenate 15 labs participated
Capelin Oil	ICES	7 chlorinated pesticides 1 PCB (aroclor 1254) (21 compounds identified)	44 labs participated 3rd ICES intercalibration sample
Mussel	ERNL/USEPA	26 PAH, total aromatics PCB (Aroclor 1254) DDE, total saturates	Accepted values based on mean results of 4 lab.

c) Other Reference Materials

I.D.	Source Agency	Certified Components	Comments
SRM 1580 (Shale Oil)	US NBS	5 PAH 2 phenols 2 quinolines	Certified concentrations of organics in shale oil
SRM 1647 (Priority pollutant PAH)	US NBS	16 PAH	Certified concentrations of PAH in acetonitrile
SRM 1649 (Urban dust)	US NBS	5 PAH	Certified in urban dust

Table 10.3

Addresses of Reference Material Suppliers

Analytical Quality Control Services
International Atomic Energy Agency (IAEA)
Laboratory Seibersdorf
P.O. Box 590
A-1011 Vienna, Austria

International Council for the Exploration of the Sea (ICES)
Palaegade 2-4,
DK 1268
Copenhagen, Denmark

Marine Analytical Chemistry Standards Program
National Research Council of Canada (NRCC)
Division of Chemistry
Montreal Road
Ottawa, ON
K1A 0R9

National Bureau of Standards (NBS)
Office of Standard Reference Materials
Washington, DC 20234
USA

National Institute for Environmental Studies (NIES)
Japan Environment Agency
P.O. Yatabe Tsukuba
Ibaraki 300-21
Japan

National Water Research Institute
Canada Centre for Inland Waters (CCIW)
867 Lakeshore Road
P.O. Box 5050
Burlington, ON
L7R 4A6

NOAA/BLM
Northwest and Alaska Fisheries Centre
Environmental Conservation Division
2725 Montlake Boulevard East
Seattle, Washington
98112

ERNL/USEPA
Quality Assurance Branch
U.S. Environmental Protection Agency
Cincinnati, Ohio
45268

US Geological Survey (USGS)
National Center
Stop 972, Reston, VA 22092
USA

a) **Documentation of analytical measurements should provide information sufficient to support all claims made for all the results.** Documentation requires all information necessary to (a) trace the sample from the field to the final results, b) describe the methodology used, c) describe the confirmatory evidence, d) support statements about detectability, e) describe the Quality Assurance program and demonstate adherence to it, and f) support confidence statements for the data. Methods of determining detection limits, accuracy and precision must be clearly stated and outlined.

b) **Laboratory records should be retained in a permanent file for a length of time set by government, other legal requirements, or the employing institution, whichever is longer.**

c) **Measurement results should be expressed so that their meaning is not distorted by the reporting process.** Reports should make clear which results, if any, have been corrected for blank and recovery mesurements and calibration conversions. Any other limitations should also be noted. **As a minimum, the formula used should be given.**

d) **Data should be reported only to the number of significant figures consistent with their limits of uncertainity.** State the uncertainty to two significant figures and the reported value to the last place in the uncertainty statement.

e) **When appropriate, the relationship between individual sample values, blanks, recoveries, and other supporting data should be shown.** If possible, and within the scope of desired resuls, a number of mesurements sufficient for statistical treatment should be made. When this is not the case, an explanation is necessary including complete details of the treatment of the data. If average values are reported, an expression of the precision, including the number of measurements, must be included. **The minimum requirements for data to be used for statistical comparisions is the mean, standard deviation and number of replicates.**

f) **Data should be reported using S.I. units, where appropriate.**

g) **If a published methodology is used, it should be cited.** Any modification, as well as any new methodology or new approach to the making of measurements or interpreting the results, must be described in detail, including test results and details of its validation. Instrumentation should be described.

The cost of documenting and reporting will vary with the interpretive detail required. An allowance of 10-20% of the total budget should be made for reporting.

11. SELECTION, MAINTENANCE AND COLLECTION OF ORGANISMS FOR BIOASSAYS

11.1 Selection of Species

The review of the literature (Volume 1, Section 7) revealed that no single species is suitable for all bioassay tests. Nevertheless certain criteria can be established to help select appropriate species. These criteria are summarised in Figure 11.1 and outlined below:

The investigator should develop a set of criteria to be met and then select the most appropriate organism. As it is unlikely that any one species will meet all of the requirements, it is important to consider using two or more species. Some important questions to answer in selecting suitable organisms are:

(1) What is the reason for the bioassay? If the bioassay is for regulatory compliance then fish or a required organism must be used.

(2) In what environment is the material expected to have the most serious impact? An ecologically important or economically important species living in that environment (e.g., pelagic - fish; benthic -crustacea), should be chosen.

(3) What effect is to be examined? A species in which this effect is most easily observed or interpreted (short-term lethal effect, long term effect, bioaccumulation, sublethal short effect - fish, crustacea, bivalves, fish respectively), should be chosen.

(4) Is the test species suitably sensitive to the substance under study?

(5) How well documented is the life history of the selected test species? Pick a species that has a well documented life history over one that does not.

(6) How easily maintained is the species in the laboratory?

(7) How widely is the species distributed within the environment? Meeting this criterion allows differing laboratories to use the same test species.

A major criterion is the end use or goal: if the biological assay is to be conducted for regulatory compliance **then the choice will be dictated by the regulatory agency.** It has been strongly recommended that the Threespine Stickleback (Gasterosteous aculeatus) be used as the test organism in the Arctic, since it is small (5 cm long) and hence convenient for laboratory use, lives in salt water and is found on the arctic coast of North America (Wong, 1982). Although this species is recommended as a standard assay organism alternate species could be used.

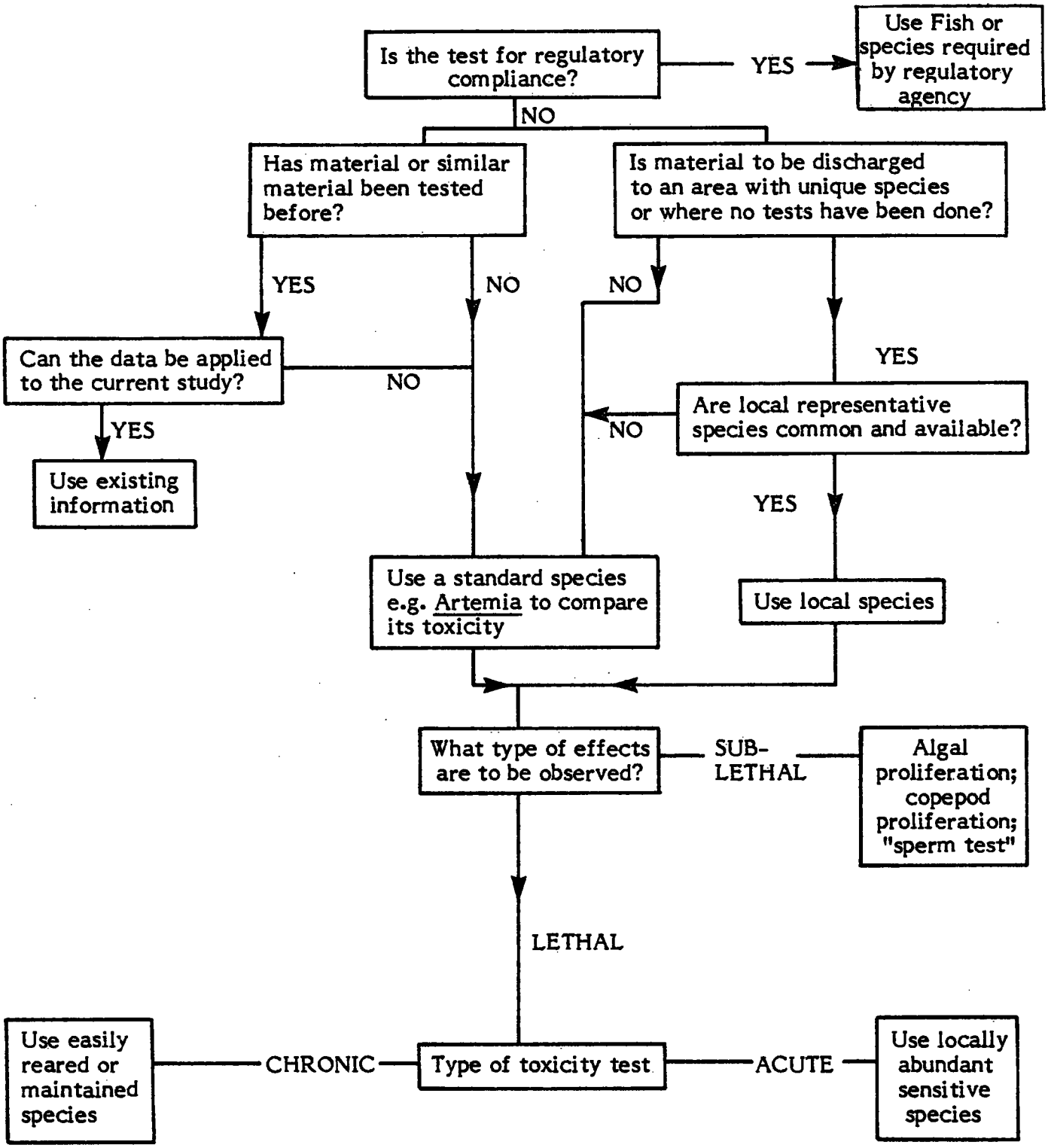

Figure 11.1. Flowchart for selecting test organisms for bioassays.

The sensitivity of the test organism is important. If only fish are used then under-estimations of the toxicity of substances could be obtained as fish are in general less sensitive than many other organisms to contaminant inputs.

Using either locally available ecologically important or economically important species as test organism is also a consideration as materials may be discharged into a new environment having a unique assemblage of species on which no bioassay tests have been conducted. In order to have an idea of the significance of the resulting data, they could be compared against results of a similar bioassay in which fish are used. In addition, reference toxicants can be used to provide a standard, facilitating comparisons and intercomparability among different laboratories.

The expected site of impact is also an important factor. A reason for using other species in addition to the stickleback or other fish which live in the pelagic environment is that the water column may not be as seriously impacted as the benthic environment. Hence it is important to consider using a benthic-dwelling organism as the test species when assays are being conducted on solid material that will rapidly settle out.

Finally, the type of effect that is to be investigated plays an important role in species selection. If short-term effects are to be studied then tests of lethality can be conducted (e.g., acute toxicity tests). In this instance, it is important to use a species that has easily recognisable indications of death or approaching death. Both fish and crustacea meet this criterion in that either opercula movement in the fish ceases or the fish turns on its back or both while in crustacea it is often easy to observe in ceasation in the movement of the animal or its appendages. The same can not be said of molluscs or enchinoderms. However, if long term effects are to be studied then molluscs may be better suited. For example, bivalves are well suited for studying the bioaccumulation of pollutants. Observations of the long-term effects over the lifetime of an organism are most easily conducted using relatively short-lived species such as copepods. Algae are also suitable for this type of work. If short-term, sublethal physiological effects are to be examined (e.g., enzyme inhibition or induction) fish are probably the most suitable test organism as physiological responses to pollutants are well documented in these organisms as compared to the invertebrates.

11.2 Collection and Maintenance of Organisms for Bioassays

11.2.1 General Considerations

a) Collection and Handling

Since intact, healthy organisms are of prime importance for successful laboratory maintenance and subsequent bioassays, trauma during collection and any consequent handling must be reduced to a minimum. A detailed discussion of methods for sampling the benthic environment is given in Sections II-3 and II-4 of volume 1 as well as in Holme (1964).

Commonly, a variety of biological dredges and trawls are used for exploratory qualitative sampling of the epifauna. These devices skim the surface of the seabed and are effective for collecting larger or scarcer epifauna and fast-moving animals such as bottom living fish, cephalopods and crustaceans. Dredging and trawling in arctic waters is, of course, limited to open water conditions. A detailed explanation of the different types of gear as well as techniques and methods for their use is provided in Volume 1, Section II-3.2.1.

A grab sampler is the conventional and best means for qualitatively sampling benthos on soft bottoms. A description of the gear, advantages and disadvantages of the various grabs and factors to consider in grab selection are given in Volume 1, Section II-3.3.1 and in sections 3 and 5 of this Guide. Factors associated with arctic sampling tend to favour the van Veen grab for general use.

Diving is probably the best technique for investigating the macrobenthos of hard or gravelly substrates which cannot be adequately sampled by dredge or other means (see Volume 1, Section II-4 for further details). Hand collection by divers is a very effective means of obtaining specimens in good condition for laboratory studies. Suction devices combining an airlift and a pump can be used by divers for the careful collection of non-attached forms and for sampling the infauna in soft bottoms. This method of collection can yield animals which are not visibly injured and which appear to be in good physiological condition (see Schneider and Hanes, 1981). Divers can also assist in the collection of fish.

The collection of pelagic organisms, including phytoplankton, zooplankton and fish is best accomplished by the use of various types of nets.

Baited traps are often ideal for collecting the smaller, faster-moving members of the epibenthic fauna such as amphipods, isopods and decapod crustaceans.

Sediment samples collected with grabs or dredges must be processed to remove the organisms. Extraction of the infauna usually involves emptying the contents of the dredge or grab onto a sieving table whereby a gentle stream of water from a sprinkler system or hose removes the surrounding sediment, leaving the specimens intact. Washing the sample through a nest of screens of various mesh size will aid recovery of smaller organisms.

b) Maintenance

The successful laboratory maintenance of organisms largely depends on how well natural environmental conditions can be simulated. The four essentials of successful maintenance are:

1) the water (medium) used,
2) the management of its life-supporting qualities,
3) adequate nutrition of the organisms, and
4) proper equipment.

Any changes in water quality must be counteracted by mechanical, biological or physico-chemical treatment. Kinne (1976) gives a detailed description of water quality management and technology in relation to the cultivation of marine organisms.

There are two basic categories of aquatic culture systems: still-water and running-water (open or closed). Only bacteria, unicellular plants and a variety of small invertebrates can be maintained in still water. Flow through (open) systems are advantageous but are not absolutely necessary as long as the water is filtered and water quality is maintained. Recirculating (closed) systems (see King, 1975 and Kinne, 1976) offer easier control and monitoring of many environmental parameters and they can be established far from a salt water source.

In the laboratory the animals should be allowed to acclimate before they are used as test organisms. During maintenance, overcrowding of the test organisms must be avoided. Careful attention should be paid to maintaining a suitable water temperature, particularly in arctic species which may have a very narrow range of temperature tolerance.

Adequate nutrition and disease control also constitute major problems in the laboratory maintenance of organisms. Phytoplankton stocks can be used to maintain

certain filter feeders such as pelecypod species. Guillard (1975) describes relatively simple and reliable methods for the culture of marine phytolankton species for feeding marine invertebrates. Mixtures of phytoplankton and larger zooplankton can provide an adequate food supply for other species of bivalves and barnacles. A beef heart extract (McCammon, 1975) has been used with great success as a supplemental food for filter feeders which cannot subsist long on plankton alone and for other invertebrates that prefer a more solid diet.

Disease causing microorganisms can be inhibited or eliminated by the use of antibiotics. Streptomycin and penicillin are used most frequently, streptomycin being better suited for the crude culture of marine invertebrates because it is a wide spectrum antibiotic with a relatively long half-life and is not readily deactivated at alkaline pHs. D'Agostino (1975) discusses the application of various types of antibiotics in the culture of invertebrates. It is usually a good idea to include a fungicide in antibiotic mixtures to control fungal growth.

A more detailed discussion of the collection and maintenance of various species to be used as test organisms in bioassays can be found in Volume 1, Sections III-7.3 and 7.4.

11.2.2 Recommended Methods

a) **Microalgae**

a.1 Collection and Handling

Sampling the phytobenthos, in particular, benthic marine microalgae, can be accomplished by collecting sediment samples with a corer and then separating the different types (see Volume 1, Section II - 5.2.2). Usually the collection of the pelagic forms of the microalgae in the phytoplankton is carried out by using nets. The microalgae are then carefully separated from the plankton tow samples. Also, pelagic microalgae can be obtained from plant fragments or other debris in the water or by sampling naturally ocurring blooms.

After collecting the crude sample, the next step is to obtain uni-algal clone cultures and then bacteria-free cultures. Ukeles (1976) defines a variety of methods for isolating representatives of single algal species and for the purficiation of algal species from contaminating organisms as well as media preparation, sterilization and subculturing.

a.2 Maintenance

Conventional culture methods for marine unicellular algae involve the inoculation of solid or liquid media dispensed into various types of culture containers, including test tubes, flasks or carboys.

Antibiotics alone or combined with detergents are often used to purify contaminated cultures, as a direct treatment or in conjunction with some other purification technique.

The nuitritional requirements for microalgae include a varying number of mineral elements (probably 15 to 20), an inorganic nitrogen source, trace metals, a carbon source, vitamins and Na, K, Mg and Ca (see Ukeles, 1976 for more details).

Ukeles (1976) discusses the physical aspects - illumination, temperature, salintiy and pH - which are necessary for the culture of microalgae.

a.3 Suggested Arctic Species

Phaeodactylum tricornutum, Skeletonema costatum and Dunaliella tertiolecta are three of the most common microalgal species which are used as assay organisms. If these are not available from arctic waters then closely related species should prove to be acceptable bioassay organisms. As part of the Baffin Island Oil Spill (BIOS) Project in situ studies were conducted to assess the effects of oil and dispersed oil on primary productivity of the ice algae community (Cross, 1982; Cross and Martin, 1983). The ice algal community was dominated by pennate diatoms, in particular, Nitzschia grunouri and N. frigida.

b) Ciliated Protozoa

b.1 Collection and Handling

Protozoan organisms, e.g. ciliates, are commonly collected in sediment samples taken by corers or grab.

b.2 Maintenance

Kinne (1977) has tabulated the approximately twenty species of marine ciliates which have been cultured, the media they were grown in and the food they

were given. Ciliates will adapt to various artificial seawaters when adjusted to the original salinity. Ciliate cultures can be maintained in clean and sterile Petri dishes, test tubes or various flasks. Provasoli (1977) discusses purification of ciliate cultures with various antibiotics, as well as various media which may be used for isolation and growth. Most ciliates feed phagotrophically (ingestion and subsequent digestion of solid particles of food) on bacteria but in suitable artificial media they can grow osmotrophically (absorption of organic substances in solution) on dissolved nutrients.

b.3 Suggested Arctic Species

Cristigera spp., common bacterivorous, sediment-living marine ciliates have been used as toxicity indiators for heavy metals (Gray and Ventilla, 1973; Gray, 1974). This ciliate, or a closely related species, should be an acceptable bioassay organism.

c) **Nematodes**

c.1 Collection and Handling

These worms are usually collected by dredge or grab. They can be removed from the sediment in the field using a gentle stream of water and sieving screens or can be carried to the laboratory in the sediment which acts as a temperature buffer. Either way, avoid temperature extremes by transporting them in insulated coolers.

c.2 Maintenance

Kinne (1977) has tabulated culture media and food sources for those marine and brackish nematodes which have been cultured. These worms have been grown on fungal mats in liquid culture as well as in a variety of agar media.

In the natural environment most nematodes feed on decaying or living microorganisms (bacteria, fungi) or microalgae associated with sediment habitats. Many free-living nematodes are carnivorous and feed on small metazoan animals, including other nematodes. In laboratory cultures bacteria, yeasts, flagellates, fungi, ciliates and diatoms have been used as food sources for nematodes.

c.3 Suggested Arctic Species

The potential role of marine nematodes as indicators of water-quality and pollution load has not been investigated to any great extent. Adequate culture methods remain to be worked out for most marine and brackish nematodes. However, Samoiloff et al. (1980) have described a long-term toxicity assay for aquatic contaminants using the nematode, Panagrellus redivivus and Tietjen and Lee (1984) have used two species of free-living nematodes as bioassay organisms for pollutant-contaminated estuarine sediments. Any abundant local arctic species, preferably related to the aforementioned worms, should be considered as possible test organisms for bioassays.

d) Polychaetes

d.1 Collection and Handling

Like the nematode worms, polychaetes are commonly collected by dredge or grab samplers. These worms can be separated from the sediment in the field or can be transported to the laboratory in the sediment which acts a a temperature buffer. A gentle stream of water (it should be the same temperature as the water from which the sample was collected) and sieving screens facilitate the removal of intact specimens. Avoid temperature extremes by transporting the worms in insulated coolers. The polychaetes, Pectinaria hyperborea and Brada villosa, have been collected for use in bioassays from near Barrow, Alaska using an airlift operated by a SCUBA diver (Schneider and Hanes, 1981). They were transferred immediately to buckets of freshly collected seawater to prevent thermal shock. The polychaete Melaenis loveni, has been obtained for use in toxicity tests by divers below the ice from the Prudhoe Bay area of the Beaufort Sea (Nortec, 1981).

d.2 Maintenance

It is preferable, although not always practical to place each specimen in its own container. Polychaetes are generally maintained in small enclosures which should be non-toxic and thoroughly cleaned; finger bowls and petri dishes are most commonly employed. Dean and Mazurkiewicz (1975) discuss in some detail the culture of polychaetes, both adults and larvae. An open sea water system, usually

with a filtering system, is most commonly used for maintaining large numbers of adult polychaetes. Closed systems can also be used for maintenance and range from standing water kept in motion via aerators, stirrers or shakers to recirculating water systems with filters. The water should be changed frequently, especially if food is added. Adequate temperature control is a must for the successful maintenance of polychaetes.

Some form of substratum often enhances maintenance; a layer of detritus and/or fine sediment works well. Several artificial substrates, absorbent cotton and the mineral, cryolite, have been used successfully. Artificial burrows of plastic or glass tubing can be used to maintain nereids, arenicolids, some spionids and glycerids.

Many species of worms can be kept for short periods without food; extended periods without the addition of food results in a reduction in body size. The natural foods which have been given to polychaetes include detritus, cultured phytoplankters (diatoms and flagellates), fresh or dry macroscopic algae, spinach and animals. Liver powder, powdered alfalfa and commercial fish food comprise the artificial foods which have been used.

Polychaete embryos and larvae also can be maintained in the laboratory. The larvae may be procured either by the laborious process of isolating them from plankton and meiobenthic samples or the more ideal method of obtaining early developmental stages by inducing the adults to spawn naturally in the laboratory. Dean and Mazurkiewicz (1975) detail the various culture systems for maintaining larval forms, both pelagic and nonpelagic, as well as appropriate food sources.

d.3 Suggested Arctic Species

Polychaetes used in previous studies, Petinaria hyperborea and Brada villosa (Schneider and Hanes, 1981) and Melaenis loveni (Nortec, 1981), would be logical species to consider. Any local species which is available in sufficient numbers could qualify as a test organism for bioassays.

e) Echinoderms

e.1 Collection and Handling

Many adult echinoderms, especially deep water forms and those which bury themselves, are collected by dredging. In shallower waters, asteroids and echinoids are collected by grab samplers or by diving. For example, the sea urchin Strongylo-

centrotus drobachiensis has been collected by divers in 7 metre water depths off the western shore of Ragged Island, a site near Cape Hatt, Baffin Island (Engelhardt et al., 1983; Mageau and Engelhardt, 1984). Specimens should be kept cold and damp during transportation.

e.2 Maintenance

Most echinoderms can be kept in aerated aquaria or tanks, often in closed sea water systems. However, if the specimens are to be kept for any length of time an open-water system is virtually the only way to maintain them in healthy condition. Direct sunlight apparently is undersirable or even harmful to most species; dim daylight or dim artificial light, simulating natural diurnal changes, is best. Avoid overcrowding.

Echinoids (sea urchins) are omnivorous opportunists which in the field feed on multicellular algae or on living or dead animals. In the labortaory, they do well on a variety of sea weeds but can also be fed spinach, lettuce, meat from bivalves, annelids, crustaceans and fish, trout food, dog food, frozen shrimp and boiled egg. The food source for asteroids (starfish) depends on their feeding types. Deposit feeders (e.g. Patiria miniata) extrude their stomach and take up food items from the sediment surface. Suspension feeders (genera Henricia, Linckia, Echinaster, Porania) can be maintained on fine particulate matter or phytoplankton. Predatory starfish can be maintained for long periods on small pieces of shellfish as well as the meat of annelids, crustaceans and fish. Many other echinoderms can be kept for several weeks without any food at all if they are kept at cold temperatures.

As part of the Baffin Island Oil Spill (BIOS) Project at Cape Hatt (N.W.T.) the green sea urchin, Strongylocentrotus drobachiensis, was used as one of three test organisms to assess behavioural responses in benthic invertebrates to dispersed crude oil (Engelhardt et al., 1983; Mageau and Engelhardt, 1984). They were maintained in a flow-through seawater system on site at Cape Hatt in 50 L aquaria at temperature and salinity conditions similar to waters from which they were collected. Subdued natural lighting was used and seaweed (Laminaria sp.) and broken shell material were supplied regularly during the pre-exposure period. The urchins were held in test tanks without sediment at densities that did not exceed field densities.

The larvae and eggs of sea urchins can be used to assay water quality. Gametes of echinoderms are obtained after natural spawning, after gonad dissection or after artifically induced spawning. Kinne (1977) discusses in great detail the rearing of echinoderm larvae as well as environmental and nutritional requirements.

e.3 Suggested Arctic Species

Any local species which can be obtained in sufficient numbers and which withstands the stress of collection and handling is a candidate for use as a test organism. As mentioned in the preceeding section, Strongylocentrotus drobachiensis, has been used as one of three test organisms to assess behavioural responses in benthic invertebrates to dispersed crude oil (Engelhardt et al., 1983; Mageau and Engelhardt, 1984) and therefore should be considered as a possible test organism for any future toxicity studies.

f) Crustaceans

f.1 Copepoda

f.1.1 Collection and Handling

Commonly, pelagic copepods are collected using plankton nets. For example, Percy and Mullin (1975) collected the herbivorous copepod, Calanus hyperborea, by means of vertical plankton tows through leads in the Beaufort Sea pack ice. Benthic copepods can be obtained by grab samplers or by divers using an airlift.

f.1.2 Maintenance

Culture methods for benthic and planktonic marine copepods are given in Kinne (1977). Usually natural sea water, filtered and unfiltered, is used as the culture medium, though artificial sea water can also be used. Sometimes antibiotics and chelating agents (EDTA) are added to the medium. Benthic copepods should have the water changed every 3-4 days. Some survive with no food for long periods while others utilize bacteria or a variety of unicellular algae. The culture container for planktonic copepods should be slowly rotated or aerated. There should be frequent water exchange or a recirculation or flow-through system should be used. Excessive handling and too much contact between the copepods and culture-vessel walls should be avoided. Algae such as Dunaliella sp., Isochrysis galbana, Phaeodactylum tricornutum and Skeletonema costatum qualify as good diets for planktonic copepods.

f.1.3 Suggested Arctic Species

The copepod, Calanus hyperborea, was used previously (Percy and Mullin, 1975) as a bioassay organism and should be considered in any future studies. Any local species which is available in sufficient numbers could qualify as a test organism.

f.2 Mysidacea, Isopoda, Amphipoda

f.2.1 Collection and Handling

The benthic forms of these crustaceans can be collected using a variety of gear and techniques, including trawls, dredges, grabs, baited traps and divers. The best methods for obtaining intact, healthy specimens are by means of baited traps or divers, equipped with an airlift. The specimens can be transported to the laboratory in plastic bags or containers filled half and half with water and air. Mysids can be collected with plankton nets and transported to the laboratory in buckets of sea water.

Several species of amphipods and isopods have been collected from the western Beaufort Sea for use as bioassay organisms (Percy, 1975; Percy and Mullin, 1975; Percy et al., 1978). Benthic amphipods, Onisimus affinis, Corophium clarencense, Atylus carinatus and Gammarus oceanicus were readily collected in modified minnow traps lined with fine nylon screen and baited with fish. This method not only yielded adequate numbers of animals in completely undamaged condition but also was the only feasible method of collection through heavy winter ice. C. clarencense and A. carinatus also were screened from mud samples obtained with a Petersen grab or a light-weight dredge. The amphipods were transported to the laboratory in insulated coolers. The benthic isopods, Mesidotea sabirica, Mesidotea sabini and Mesidotea entomon, usually were obtained by otter trawl. In addition, M. entomon and M. sabirica were collected in baited traps. M. sabini which is a burrowing form was not readily caught in baited traps but was obtained by bottom trawling. These isopods were frequently held 1 or 2 days in large polyethylene tanks of aerated seawater before being transported by air in two gallon jugs of cold sea water (Percy et al., 1978).

In a Northern Technical Services study (Nortec, 1981) amphipods (Onisimus sp. and Boekosimus sp.) from the Prudhoe Bay area of the Beaufort Sea were collected in baited traps which consisted of 18L plastic buckets with three screened

hatches. The bait used was either salmon or shrimp. In the same study isopods (Saduria entomon) were collected by hand by divers below the ice and mysids (Mysis sp.) were colleced with plankton nets in shallow waters and transported to the laboratory in 18L buckets of sea water.

Schneider and Hanes (1981) obtained benthic infaunal species (amphipods, Asceroides latipes and Pontoporeia femorata; isopos, Saduria sabini) from near Barrow, Alaska for toxicity tests by using an airlift operated by a SCUBA diver. Epibenthic species (amphipods, Anonyx nugax and Boeckosimus affinis; mysid, Mysis littoralis) were collected with amphipod traps baited with sardines.

f.2.2 Maintenance

These organisms can be maintained in small enclosures which are frequently not aerated, in non-running but regularly renewed water and in larger enclosures (aquaria, fibreglass tanks) in which the water is usually aerated and often recirculated and filtered. Many benthic isopods and amphipods require or prefer the provision of hiding places or substrata such as sand or gravel. Most forms prefer dim light or quasi darkness. Benthic forms have been fed detritus containing bacteria, fungi or protozoans; plants such as diatoms, Ulva lactuca, lettuce, spinach; living animals such as oligochaetes and pieces of molluscs, crustaceans and fish. In addition, various commercial fish foods have been used successfully. Planktonic forms have been fed a variety of phytoplankton, copepods, rotifers, Artemia salina and organic detritus.

As mentioned in the previous section several species of amphipods (Gammarus oceanicus, Onisimus affinis, Corophium clarencerse, Atylus carinatus) and isopods (Mesidotea entomon, Mesidotea sabina, Mesidotea sibirica) have been collected from the western Beaufort Sea (Percy, 1975; Percy and Mullin, 1975; Percy et al., 1978). Sometimes these organisms were held in large tanks of Instant Ocean Seawater at a temperature and salinity that approximated their natural habitat. The water was continuously aerated, filtered and changed regularly. In addition, some of these crustaceans were shipped south to the Arctic Biological Station in Quebec and held in a high capacity refrigerated, recirculating sea water system (Instant Ocean) at a temperature of 1°C and a salinity of 15 - 17 °/ oo. In both cases the organisms were fed Tetramin fish food. Both these methods apparently maintained these organisms in healthy condition. However, the duration of the maintenance is not stated.

The Northern Technical Services study (Nortec, 1981) utilized a portable laboratory which was located on the sea ice in winter and then relocated on shore prior to spring breakup. The laboratory contained six plywood troughs with flowing sea water and drainage systems. The sea water was pumped into the laboratory through an intake line passing first into large inflatable tanks (air berm) where some settling of suspended material occurred before the water was used for cooling, holding organisms and bioassays. The amphipods (Onisimus sp. and Boeckosimus sp.), isopods (Saduria entomon) and mysids (Mysis sp.) were maintained without food in 50 L tanks set in two cooling troughs in the laboratory. They were held for 48 hours prior to testing with the holding water being changed approximately every 6 hours. This method apparently was successful for maintaining these organisms in healthy conditions. Few mortalities (less than 2 percent) were observed in their handling and holding. However, the length of time these crustaceans were held in the laboratory is not given.

Schneider and Hanes (1981) maintained their bioassays organisms (amphipods, Asceroides latipes, Pontoporeia femorata, Anonyx nugax, Boeckosimus affinis; isopod, Saduria sabini: mysid, Mysis littoralis) at 32 °/oo saliniy, -1.0 °C and in the dark in Percival constant temperature incubators under static water conditions. The holding containers were not aerated but the oxygen content was monitored and did not fall below 75 % of saturation. The organisms were not fed and were usually held for no more than two to three weeks to avoid problems associated with long-term maintenance. Survival was excellent under these maintenance conditions and the animals appeared to be in good health for periods exceeding one month.

f.2.3 Suggested Arctic Species

Any of the species used in the three studies which were cited previously in this section should be considered as bioassay organisms for any future studies. These include the amphipods, Onisimus (= Boeckosimus) affinis, Corophium clarencense, Atylus carinatus, Gammarus oceanicus, Asceroides latioes, Anonyx nugax, and Pontoporeia femorata; the isopods, Mesidotea sabirica, Mesidotea sabini, Mesidotea entomon; and the mysid, Mysis littoralis. In addition, in situ studies (Baffin Island Oil Spill Project) have been carried out to assess the effects of oil and dispersed oil on under-ice amphipod communities (Cross, 1982; Cross and Martin, 1983). The amphipods used in these experiments include the following species: Weyprechtia pinguis, Onisimus litoralis, O. glacialis, Gammarus setosus and Ischyrocerus sp.

f.3 Decapoda Natantia (Shrimp and Prawns)

f.3.1 Collection and Handling

Shrimp and prawns are collected by means of nets, trawls, baited traps or by divers. They can be transported to the laboratory in plastic bags or containers, filled half and half with water and air, preferably in insulated coolers.

f.3.2 Maintenance

Adult shrimp and prawns should be kept in aquaria and related medium-sized enclosures. Decapod larvae can be accommodated in culture dishes (water renewal every second day), compartmented tray or in culture systems in running water. These crustaceans will accept a large variety of food items ranging from plants, invertebrates and fishes to commercial fish foods. However, live foods such as Artemia salina or meat of freshly killed animals (e.g., clams, mussels, squid, crustaceans, fish) seem to be the best diet. The diets consumed by reared decapod larvae include phytoplankton algae such as species of Dunaliella, Chlorella, Isochrysis, Skeletonema, Phaeodactylum, etc., and zooplankters such as rotifers and copepods, as well as eggs and larvae of annelids, cirripedes, molluscs, echinoderms and fishes. Mixed diets are more likely to meet all nutritional requirements. Since benthic natantians such as pink and brown shrimp seek protection in sediments some kind of substratum or protective covering should be made available. Kinne (1977) gives a detailed discussion of rearing both adult and larval decapods in the laboratory.

f.3.3 Suggested Arctic Species

Apparently no Arctic natantians have been used as bioassay organisms. However, any local species which can be collected in sufficient numbers is a possible candidate for use as a test organism.

g) Bivalve Molluscs

g.1 Collection and Handling

Adult bivalve molluscs are collected by dredge, grab or diver. The adults of many species experience no adverse effects when transported to the laboratory in

containers with cold moist toweling. These organisms can also be transported in containers of cold sea water, preferably in insulated coolers. Bivalve larvae for use in bioassays can be obtained by screening living larvae from plankton samples or by the more desirable method of collecting adults and obtaining gametes from them.

In the Arctic (two sites near Barrow, Alaska) the bivalve mollusc, Liocyma fluctuosa, was collected with an airlift operated by a SCUBA diver for use as a bioassay organism (Schneider and Hanes, 1981). The clams were transferred immediately to buckets of freshly collected seawater to prevent thermal shock. The bivalves, Serripes groenlandicus and Mya truncata, were collected by divers in 7 metre water depths off the western shore of Ragged Island, a site near Cape Hatt, N.W.T. (Engelhardt et al., 1983; Mageau and Engelhardt, 1984) for use as test organisms.

g.2 Maintenance

Juveniles and adults are best maintained in the laboratory in a flowing natural sea water system (closed or open) at controlled temperatures in trays or tanks with several inches of natural substrate (Chanley, 1975; Culliney et al., 1972). The majority of bivalves are suspension feeders and normally the natural food supply in the running water is sufficient to maintain the organisms in good physiological condition. However, supplemental feeding with phytoplankton (natural or cultured) is recommended. The water in an open sea water system can be enriched with suspensions of food algae. In the laboratory bivalves have been raised on a variety of unicellular algae. The deposit feeding bivalves which obtain their food from the bottom detritus will need access to a substratum of sediment.

As mentioned previously the bivalve mollusc, Liocyma fluctuosa, was collected from sites near Barrow, Alaska for use as a bioassay organism (Schneider and Hanes, 1981). These animals were maintained in the dark in Percival constant temperature incubators under static water conditions at -1.0°C and 32 °/oo salinity. Though the holding containers were not aerated the oxygen content of the water was monitored and did not fall below 75% of saturation. Animals were not fed during maintenance but were not held for more than two or three weeks. Survival was excellent under these maintenance conditions.

The two bivalve species (Serripes groenlandicus and Mya truncata) collected from near Cape Hatt, N.W.T. were held in a flow-through seawater system on site at Cape Hatt in 50L aquaria at temperature and salinity conditions similar to those found in the offshore waters where they were collected (Engelhardt et al., 1983;

Mageau and Engelhardt, 1984). The bivalves were maintained under subdued natural lighting conditions and were acclimated for at least seven days. S. groenlandicus were kept in a 10 cm layer of washed and sifted coarse beach sand while Mya truncata were held without sediment in the tanks. Holding densities did not exceed field densities.

Bivalve larval stages are often used as test organisms for bioassays because they are more sensitive than the adults to environmental conditions. Loosanaff and Davis (1963) and Kinne (1977) contain detailed discussions of the laboratory rearing of bivalve larvae. Larvae can be obtained by screening them from plankton samples but are most often obtained from adults which are collected in the field, conditioned in the laboratory and then stimulated to spawn. Larvae are kept in a variety of enclosures (cylindrical in shape), including Pyrex glass beakers, lobster jars, fibreglass containers, etc. The culture water should be renewed every second day unless running water is used. Use filtered seawater for maintianing the larvae. No food sources other than a variety of unicellular algae such as Isochrysis galbana, Monochrysis lutheri, Dicrateria inornata, Chlorococcum sp., Cyclotella nana, etc. have been found satisfactory for maintaining the larvae. Antibiotics, fungicides and germicidal ultraviolet rays are used to treat the sea water for combating microbial growth.

g.3 Suggested Arctic Species

Since the dominant species will vary considerably with location, any local bivalve species which can be collected in sufficient numbers is a possible candidate for use as a bioassay organism. If several sites are being monitored, a common species may not be available and/or abundant at all sites.

Several species of Arctic bivalves have already been used in toxicity studies and should therefore be considered for use in future studies. Liocyma fluctuosa collected near Barrow, Alaska, has been used as a bioassay organism (Schneider and Hanes, 1981). As part of the Baffin Island Oil Spill (BIOS) Project two species of bivalves, Mya truncata and Serripes groenlandicus, were used as test organisms to assess the behavioural and physiological responses of benthic invertebrates to dispersed crude oil (Engelhardt et al., 1983; Mageau and Engelhardt, 1984). In addition, such bivalves as Portlandia arctica, Astarte sp. and Macoma sp. are possible candidates for use in toxicity tests.

h) Fish

h.1 Collection and Handling

The collection of fish is best accomplished by the use of various types of nets or with the assistance of divers. Fish must be handled gently at all times, especially when being transferred. They can be transported in insulated containers of cold freshly collected sea water.

Several species of fish found in the Arctic have been collected for use in bioassays. The fourhorn sculpin, Myoxocephalus quadricornis, has been collected from near the entrance to Eskimo Lakes (western Beaufort Sea) by scooping them up individually in beakers (Percy and Mullin, 1975). In another study (Nortec, 1981) five species of fish (the fourhorn sculpin, Myoxocephalus quadricornis; the broad whitefish, Coregonus nasus; the Arctic cod, Boreogadus saida; the Arctic cisco, Coregonus autumnalis and the saffron cod, Eleginus navaga) were collected from the Prudhoe Bay area. In the winter a few fish were collected either by hand by divers below the ice or by a fyke net deployed by divers. However, the majority of the fish were collected in the summer and were captured in fyke nets deployed by the divers. Two other species of fish (the Arctic char, Salvelinus alpinus and the least cisco, Coregonus sardinella) were caught frequently but were too large to test in the 50L aquaria used in this study.

h.2 Maintenance

Fish must be acclimated prior to a bioassay, preferably for several weeks. During the acclimation period, they should be actively feeding. Acclimation tanks and accessories must be made of non-toxic materials: glass, stainless steel, or aluminum when organics are important, polyethylene, PVC or polypropylene when metals are important. These tanks should be located away from physical disturbances and preferably separated from test vessels. A constant flow of water through the holding tank is necessary during acclimation. The water should be aerated or oxygenated, cooled and filtered. The photoperiod should probably duplicate the natural one. The tank must be kept clean by periodic siphoning of settled material. To prevent overcrowding there should be at least one litre of water for every 10 grams of fish in the holding tank (Sprague, 1973). A daily inspection for unhealthy fish should be carried out. Water quality (e.g., pH, salinity, temperature and

dissolved oxygen) should be monitored. The holding tanks need to be disinfected between batches of fish. Fish should be fed at least twice a day; various types of commercial fish food are available (Kinne, 1977). Bacterial contamination and diseases can be controlled with the use of antibiotics, fungicides and by ultraviolet irradiation of the water. Wong (1982) gives a complete description of the procedures involved in conducting a fish bioassay.

As previously mentioned several species of fish found in the Arctic have been used as biassay organisms. Myoxocephalus quadricornis (fourhorn sculpin) have been maintained in a recirculating seawater system at a temperature and salinity regime approximating that of its natural habitat (Percy and Mullin, 1975). In a second study (Nortec, 1981) a portable laboratory was used which was located on the sea ice in winter and then relocated onshore prior to spring breakup. The laboratory contained six plywood troughs (plywood reinforced with fibreglass) with flowing seawater and drainage systems. Two troughs were used for holding organisms. Seawater was pumped into the laboratory through an intake line passing first into large rubber inflatable tanks (air berm) where some settling of suspended material occurred before the water was used for cooling, holding organisms and bioassays. Water was pumped from the air berm into the laboratory or into two large holding tanks. The fish (the fourhorn sculpin, Myoxocephalus quadricornis, the broad whitefish, Coregonas nasus; the Arctic cod, Boreogadus saida; the Arctic cisco, Coregonus autumnalis and the saffron cod, Eleginus navaga) were placed in these large holding tanks after collection. They were not fed but some food was introduced with the ambient seawater. Prior to testing, fish were transferred to a holding trough in the laboratory and held for 48 hours without food. Both these methods apparently maintained the fish in healthy condition. However, the duration of maintenance was not given in either study. Pool mortalities in the second study (Nortec, 1981) varied from 2 to 10 percent, fluctuating with alterations in the holding water salintity as weather and currents changed.

h.3 Suggested Arctic Species

Any of the fish species used as bioassay organisms in previous studies should be considered for use in future studies. These include the following species: the fourhorn sculpin, Myoxocephalus quadricornis; the broad whitefish, Coregonus nasus; the Arctic cod, Boreogadus saida; the Arctic cisco, Coregonus autumnalis and the saffron cod, Eleginus navaga. Rainbow trout have been the standard fish for bioassay

work on drilling fluids disposed offshore in the Arctic even though they are not an indigenous species (Wong, 1982). In addition, the ninespine stickleback which is widely distributed in the arctic would seem to be another logical choice for a bioassay organism. However, the threespine stickleback, Gasterosteous aculeatus, apparently is a better choice than either rainbow trout or the nine-spine stickleback for use as the test species in bioassys (Wong, 1982). Additionally, any local species which can be obtained in sufficient numbers and which can survive the stress of collection and maintenance should be considered for use as a bioassay organism.

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APPENDIX A

WORKED EXAMPLE

To help illustrate the planning of benthic environmental sampling and analysis programs in the Arctic, a sampling and analysis plan is developed for a hypothetical situation below. Exact details of logistics, equipment requirements, etc., have not been given.

REQUIREMENT:

Baseline (or background) information is needed in an area that may be subject to extensive offshore exploratory drilling for hydrocarbons from drillships and artificial islands. A contractor is asked to carry out a study at one proposed site. The objectives are given as:

1) characterize the sediments in terms of grain size (percentage sand, clay, silt) and TOC content.

2) determine total concentrations of key trace metals (Hg, Cd, Pb, Cr, Ba) in sediments.

3) determine background levels of petroleum related hydrocarbons (n-alkanes C_{12}-C_{30}, PAH).

4) determine abundance and species richness of benthic communities.

5) determine the body burdens (soft tissue) of trace metals (as above except Ba) and PAH in the most abundant benthic species.

The study is to serve as a basis for future comparisons and an on-going monitoring program: therefore the contractor is asked to employ analytical methods which will be able to quantitatively detect the trace parameters. A quality control program for trace metals and hydrocarbons must be identified and adhered to. The contractor is asked to suggest how many samples should be taken and where.

The contractor is also given the following information:

1) area of influence is expected to be within 2 km of drill site: currents (speed, direction) are unknown. The bottom depths are fairly uniform between 18-22 m within 10 km of the site.

2) Drilling at the site will be from a drillship.

3) No background information exists for the above parameters at the proposed site:

4) the site is ice covered for 9 months on average: the site is outside the zone of landfast ice.

5) closest airfield/harbour/community is 100 km distant.

6) budget for the sampling and analysis is not to exceed $250,000.

7) the study must be completed within 15 months.

A proposed sampling and analysis plan is required for review: the rationale for the study approach and choice of methods is to be given.

DEVELOPMENT OF A PLAN:

1. **Assemble Available Information and Assumptions:**

a) study area is 2 km radius circle around the proposed site.

b) depth of sediment for chemical analysis - top 1-2 cm as future comparisons will be made to detect recent input.

c) clay/silt particulates should be the focus of analysis as any future contamination will be associated with this size range.

d) centre of drillsite can be regarded as a point source of any waste discharged: concentrations of discharged contaminants likely to decrease exponentially with distance from the source.

e) benthic body burdens and species richness of benthic communities will vary seasonally.

f) no seabottom structures in the area on the basis of hydrographic charts: bottom appears relatively smooth.

2. **Sampling Strategy**

a) goal is descriptive: however the site will be subject to future contaminant inputs and the survey will serve as the basis for a future monitoring (analytical) studies:

Therefore it is recommended that the study be designed with this in mind: strategy should therefore be to utilize an optimal impact study design (Section 5.3.1) and form and test hypotheses:

b) Study Area: will be a 2 km radius circle centered at the proposed site. A nearby spatial control area will be required which is thought to be outside the expected zone of influence but with similar depth and sediment characteristics.

c) Hypotheses:

H_0 = There is no change in mean abundance (or mean value of percent change in abundance) of the benthic species assemblage in the impact area (over a time interval which includes the impact) relative to the reference area.

H_A = There is a significant change in mean abundance (or mean percent change in abundance) of the benthic species assemblage in the impact area relative to the reference area.

H_0 = There are no changes in environmental conditions (e.g., contaminant body burdens in benthos, sediment grain sizes, TOC, petroleum hydrocarbons, trace metals, depth, salinity, temperature) in the impact area relative to the reference area.

H_A = There are significant changes in environmental conditions (contaminant body burdens in benthos, sediment grain sizes, TOC, petroleum hydrocarbons, trace metals, depth, salinity, TOC) in the impact area relative to the reference area.

d) Where to Sample: nothing is known about potential distribution patterns. Therefore random sampling is best. However, can assume that greatest impact will be close to drill site. Therefore, a useful strategy would be to divide the study area into strata: an inner area of about 300 m in radius: (high impact) and an outer circle: 300 - 2,000 m: (reduced impact) samples within each area would be selected randomly: Sampling effort would be aportioned on the basis of the expected degree of impact (i.e., a more dense allocation of sites in the high impact area).

d.1 Controls: important to incorporate a control area for future comparisons: the choices are:

i) historical (i.e., cores-analyse deeper sections). This is not valid for benthos or hydrocarbons (n-alkanes will be degraded).

ii) third strata beyond 2,000 m: feasible but since actual extent of future effects is not known, effects may extend beyond 2,000 m. Therefore, this is not an optimum configuration.

iii) separate control area: a separate area with a radius of 2 km situated several km away from the study area but in water of the same depth and similar bottom features. This control is the best for future comparisons.

With a reference area as in iii) above and a stratified random sampling, statistical analysis by one-way classification multivariate ANOVA (section 5.3.1.1) would be permitted. This type of design and analysis can be used to test for seasonal changes in the abundance and species richness of benthic populations.

Samples for chemical analysis should be from sediments with a high clay/silt content. If a site is found to be mainly sand or gravel then a new site would be selected.

e) When to Sample: Since benthic community richness and benthic contaminant body burdens will vary with season, a minimum requirement is for winter/summer sampling.

Summer sampling is probably best planned for late August, winter sampling for late March or early April, as these are likely to be the most stable periods in each season in terms of weather and ice conditions and available daylight for on-ice sampling.

f) Number of Samples:

A minimum of 3 sites for each strata required and 3 replicates for both benthos and chemical parameters (Section 3.1.3.2 and 5.). Make an estimate

of sampling cost to give an approximate idea of whether two sampling trips are practical within the proposed budget.

f.1 Open water sampling:

Sampling party: 3 (see j.5)
Assume 4 days in field and 3 days travel:

Per diem for sampling party:	$1,000/day x 4 (field) $700/day x 3 (travel)	$ 4,000 2,100
Ship charter: (including fuel, food)	3 days x $4,000	12,000
Return travel to region from south	3 x $1,000	3,000
Equipment leases/rentals (SAT/NAV, etc.)		2,000
Freight		2,500
		$ 25,600
+ 20% contingency in labour and charter		4,000
		~ $ 30,000

f.2 Through the ice sampling:

Assume 10 field days required.
Assume extra equipment required, helicopter support required.

Per diem:	$1,000 x 7 (field) $700 x 3 (travel)	$ 10,000 2,100
Helicopter	30 hours x $1,000	3,000
Travel (same)		3,000
Equipment lease/rentals		4,000
Freight		4,000
		$ 50,000
+ 20% contingency		10,000
		~ $ 63,000

Therefore it should be possible to accomodate two sampling periods within the proposed budget.

f.3 For benthos:

Minimum is 3 sites per strata: however, this is a relatively large area so that a larger number of sites is desireable. Therefore suggest 4 randomly selected sampling stations in each stratum as a start, 3 replicate samples at each location.

Therefore 24 samples per area or 48 per sampling period (2 areas).

Total samples would be 96 (48 for winter and summer sampling period).

f.4 For chemical parameters:

f.4.1 Sediment

Unlike biota, chemical content of sediment will probably not vary on a seasonal basis. However, contaminant body burdens will vary: sediments in each survey need to be characterized in terms of grain size and TOC for benthos.

No. of sites as per benthos,
with three replicates per site = 48 samples (one trip only)

plus of the 24 grabs in the control and primary areas, 8 grabs would be selected at random and triplicate subsamples taken from the same grab. Therefore 16 extra samples per area would be collected (32 in total).

Therefore, total number of samples collected (48 + 32) = 80 samples for metals and hydrocarbons.

f.4.2 Biota

It is more difficult to assign a number to tissue analyses since the type of organisms are not known. If the organisms are large enough to provide enough tissue for all analyses then analyses of individual organisms could be considered. However, it will probably be necessary to pool several individual organisms (composite) to obtain sufficient tissue for both PAH and metal analyses.

A suggested sampling design could be:

A minimum of 3 individual organisms to be collected from each of 8 sites in both reference and primary study areas (more if the most abundant organisms are very small). The samples are to be selected from the 3 replicate grabs: the organisms from each grab to be pooled and treated as a single sample.

Therefore number of samples = 32 (16 per sampling period).

g) Samplers

A short armed version of the Van Veen would be used for benthos (Section 5.4.1) since through ice sampling will be required. A Birge-Ekman sampler is a good choice for chemical parameters (Section 3.2) with the Van Veen as a back-up.

g.1 Sample Requirements

Sediment: (per analysis - wet weight)

TOC	5	g
Grain size	100	g
Metals	5	g
Hydrocarbons	50	g
Total	160	g

The cross-sectional area of the Birge-Ekman will be about 500 cm^2. If the top 2 cm is collected and a density of 1.5 g/cm^3 is assumed, about 750 g (wet weight) of sediment should be available. This is more than adequate for all chemical tests.

Tissue: (per analyses - wet weight)

Metals	5	g
PAH	50	g
Total	55	g

h) Sampling Quality Control (Section 3.4)

In addition to the replication indicated, it is recommended that 8 randomly selected individual samples be split into triplicate subsamples for analyses to give a measure of the within sample variability.

i) Logistics:

i.1 On ice logistics (Section 4.3)

i) Transport to and from study area by helicopter (helicopter must be able to take study team plus gear): 2 snowmobiles and sleds to be used to move between sampling sites. These may be driven to the site or carried in a sling under the helicopter.

ii) ice hole: hot water drill: ring diameter to take short-armed version of 0.1 M^2 Van Veen

iii) lift: aluminum tripod and small hand winch for deploying grab and removing ice block.

iv) shelter: portable tent and heater required for on-ice work

v) safety: work on ice; return each night to shore base. However, enough supplies to be taken so that party can stay several days if necessary (food, communications, rifle, sleeping gear).

i.2 Ship-board logistics (Section 4.4)

Charter vessel has hydraulic winch, 3/16" wire rope, booms, davits, accommodation, some work space, large freezer. Need to supply only sampler, storage equipment and benthic processing equipment.

i.3 Freight:

Estimate gear to weigh:

500 kg in summer (750 kg on return)

1200 kg in winter (1500 kg on return).

All equipment to be shipped in labelled wooden boxes. Formalin is restricted item: must be shipped early to avoid delays and packaged separately (Guide 4.2).

i.4 Positioning (Guide 4.3):

A SAT/NAV system is recommended because of price, portability, location and the need for both winter and summer sampling. Accuracy ±100 m is adequate.

i.5 Personnel (Guide 4.5):

A minimum party of 3 would be required for type of sampling gear and sample processing requirements.

i.6 Contingencies:

The winter sampling program may not be feasible as the area is in a region of unstable ice conditions. Therefore a contingency for an extra winter trip may need to be included. Alternatively, the sampling design may need to be revised. The effect of various scenarios should be considered: i.e.,

i) no winter samples possible
ii) not possible to return to summer sites (new sites need to be selected)
iii) only some sites can be sampled
iv) only the primary area or reference area can be sampled
v) reference area needs to be moved

and a course of action decided on if any of the above situations should arise.

3. Analysis

a. Trace Metals

The first decision is that of instrumental technique which depends mainly on the sensitivity required for accurately determining the existing concentrations of these metals in the samples. Assuming that the sediments are typical uncontaminated sediments, the results of section 6.2.3 can be applied.

GF-AAS is chosen for Cd and Pb because F-AAS and ICP are not sensitive enough. The methods of Sturgeon et al (1982) supplemented by the recommendations of Slavin et al. (1983) are chosen. These methods are based on the stabilized temperature platform furnace (STPF), and matrix modifiers are used to enable direct comparison of standards with sample solutions. F-AAS is chosen for Cr and Ba because GF-AAS is too sensitive for these metals, and ICP does not offer a significant cost advantage. Loring and Rantala (1977) and Agemian and Chau (1975a) have provided suitable instrumental methods. CV-AAS is the only instrumental technique that is sensitive enough for determining Hg. The method of MacPherson et al (1982) is chosen. Preconcentration techniques for enhancing the concentrations of Ba, Cd, Cr and Pb in the sediment solutions prior to instrumental quantification are unnecessary.

a.1 Sediment Digestion

A total attack digestion is required, except in the case of mercury where a strong attack method will suffice. As discussed in section 6.2.4.1 total acid attack digestion (with HF) is appropriate for Cd, Cr and Pb, while fusion attack is necessary for Ba. Total attack methods sometimes do not completely dissolve minerals containing barium. The digestion method selected for Cd, Cr and Pb is that of Sturgeon et al (1982). The fusion method selected for Ba is that of Owens and Gladney's (1976) as modified by the recommendations of Mahan and Leyden (1983). (Chromium can also be determined in the fusion attack solutions to check the accuracy of the acid digestion procedure.) For Hg, the strong attack procedure of MacPherson et al. (1982) is chosen. It gave accurate results for Hg in the NRC reference sediments.

MacPherson et al's method can be conveniently integrated into the scheme of Sturgeon et al (1982) since the HCl & HNO_3 digestion of MacPherson et al. is very similar to that employed by Sturgeon et al in the first step of their procedure.

The samples are dried in an oven at 40°C. This temperature should not cause any significant loss of Hg. The dried sediments are disaggregated with a plastic spatula, weighed, and screened with a nylon seive (230 mesh) and the fine fraction is weighed again. This step is done to reduce the influence of grain size effects on trace metal distribution. The fine fraction should then be homogenized and subsampled for the digestions (0.5g for Ba and 1.5g for Cd, Cr, Hg and Pb).

a.2 Tissues

The expected concentration ranges can be less accurately estimated in the case of the biota. Assuming that they are identical to those given in 6.2.4.1, the same conclusions apply.

F-AAS and ICP are sensitive enough for determining Cd, Cr and possibly Pb if preconcentration is employed. Without preconcentration, GF-AAS is sensitive enough for Cd, Cr and possibly Pb. CV-AAS must be used for determining Hg.

Since the organism selected for analysis is likely to be a clam or a worm, it is assumed that the amount of tissue per sample that is available for analysis will be very small. On this basis, the instrument of choice for Cd, Cr and Pb is GF-AAS.

For determining Cd, Cr and Pb, the methods of Hinderberger et al. (1981) supplemented by the recommendations of Slavin et al (1983) are selected. A preconcentration step would be warranted if the sample concentrations are extremely low (or if severe matrix interferences are encountered). The method of Okuno et al. (1978) is selected for preconcentrating Cd, Cr and Pb; concentration factors of up to 100 should be possible with this method. The CV-AAS method selected for determining Hg is Chapman and Dale's (1978). It is a manual method that has excellent sensitivity (L.O.D. = 0.4ug/g). The use of one digestion for all of the metals is desirable, since a significant savings of time and expense may result. The method of Feldman (1974) is selected for the metals, because it is the preferred method for Hg and it compares well with the other methods that are recommended for the Group I metals. A preliminary test procedure is used to determine which variation of the procedure is the most suitable for the kind of tissue sample to be digested. In the case of benthic invertebrates, the SNP variation will probably turn out to be the most suitable, since it is safest for samples containing much lipid. Feldman's (1974)

method also has a relatively high throughput, 20 sampler/2.5 hours. This method should be compatible with the instrumental and preconcentration methods already selected for Cd, Cr, Hg and Pb. Sample handling would be a per section 6.2.3. Approximately 300mg (dry weight) per sample is sufficient for Cd, Cr, Pb and Hg.

COST $50 - $100 / sample tissue or sediment

b. Hydrocarbons

Samples from the sampling area would be expected to be pristine with low levels of petroleum hydrocarbons. Thus a major goal would be high sensitivity. For comparison of the hydrocarbon data to data from some other time (i.e. for future monitoring), high accuracy and high precision are required.

The appropriate instrumental method, indicated in tables 7.3.1 and 7.3.2 is gas chromatography with flame ionisation and/or mass spectrometric detection. Sample requirements, indicated in section 7.3.1, are for 50g samples (wet weight) per analysis. Samples should be stored frozen in screw-top glass jars with teflon lined lids, precleaned for hydrocarbons (Section 7.3.2).

b.1 Sediments

Analysis for petroleum hydrocarbons with high sensitivity and high accuracy is described in section 7.3.6 and care in sampling and analysis are emphasized. The objective to analyse for alkanes from nC_{12} to nC_{30} eliminates consideration of the low molecular weight petroleum hydrocarbons.

Sediment extraction, as recommended in section 7.3.6.2, would be by saponification in ethanolic potassium hydroxide and partition into a non-polar solvent. The appropriate fractionation method indicated (Section 7.3.6.2) for this type of analysis is gel permeation chromatography (GPC) followed by silica gel adsorption chromatography.

Analysis of the extracts by high resolution GC/FID and /or high resolution GC/MS will provide the required n-alkane and PAH concentrations and will also allow several related parameters to be calculated (odd-even predominance, pristane/phytane ratio etc., see Table 7.3.3). The utility of such parameters is discussed in volume one (Section III, 3.4.1).

b.2 Tissues

For the PAH analysis, a similar rationale to that used for objective 3) would lead to the analytical goal of high sensitivity, high accuracy and high precision. The appropriate techniques are given in table 7.3.2. This table indicates GC/FID and/or GC/MS be used for in this situation. Sample size and storage reuqirements are indicated in section 7.3.1 and 7.3.2, which recommend 50g wet weight of tissue be collected for each analysis, tightly wrapped in cleaned aluminum foil and stored frozen.

Extraction and analysis of benthic tissue samples is given in section 7.3.1.3 using similar methods that were discussed for objective 3, (petroleum related hydrocarbon). In this case however, only the PAH fractions would be analysed preferably by GC/MS, as indicated in table 7.3.12.

COST $350 - $400 / sample for PAH, n-alkanes in sediments
$250 - $300 for PAH in tissues

c. TOC Measurement: (Guide Section 7.1)

The acid digestion - dry combustion method (Section 7.1.3) is suggested for use because it provides maximum sensitivity for organic material and is more accurate. This is important in a baseline study.

COST $20 - $30 / sample

d. Grain Size

A subsample from each grab of about 100 g wet weight should be taken* -this will be drawn last. Samples should be transferred to a bag or plastic jar. Samples need not be frozen (guide Section 8.2).

Since only clay, silt, sand on a percentage basis is required, and sufficient sample should be available, a combination of a wet sieve and hydrometer sedimentation analysis is suggested. Two sieves would be used: No. 10 (2.0mm) and a No. 230 (0.0625mm). The material passing the No. 230 mesh would be subjected to

sedimentation analysis. The material retained on the 230 mesh sieve would be the sand fraction (guide Section 8.1)

COST $60 / sample

* For upper two centimeters assume sediment density wet -- 1.5g/cc: then area of sediment required: $(100/1.5X2)^{1/2}$ $6cm^2$

e. Quality Assurance: (guide Section 10; Appendix B, Page 28)

Steps in quality assurance applicable to this study should include:

i) Quality Control

- Analysis of at least one reference material for trace metals in sediment and tissue (guide Section 10.1). Materials selected should have values for all metals if possible: USGS MAG-1 is the only sediment certified for Ba.

- Reference materials should be run with each digestion batch (a minimum of 3).

- Analysis of at least one reference material for hydrocarbons in sediments (frequency as for metals).

- A number of samples (minimum 3) should be homogenized and split and sent to another laboratory for analysis (collaborative testing) -- this is especially important for PAH analysis of tissues as no certified reference materials exist.

- 10% of samples will be reanalyzed as blind replicates (i.e. samples are homogenized and split; analyst will not know the identity of the samples).

- Reagent blanks to be run with each digest set (minimum of 3).

- A set of calibration standards (at least 3 different concentrations) to be run with each set of samples for instrumental analysis.

ii) Quality Assessment

- Control charts (Shewhart) to be maintained for
  - reference materials
  - blanks
  - blind replicates

The following criteria would be used in assessing control data (Appendix B, Page B-32):

- Not more than 1 in 20 results be outside 2 standard deviations.

- A point outside 3 standard deviations will require that the problem be identified and corrected before any more samples can be run. Samples analyzed since the last control must be re-run.

- Not more than 7 consecutive results are on the same side of the mean (if so, a systematic error is suggested and the problem identified before more samples are analyzed).

- There are no regular periodic variations.

The cost of the Quality Assurance Program will be about 10-15% of the total trace metal and hydrocarbon costs.

4. **Estimated Cost:** (an estimated cost for the sampling and analysis plan is indicated below)

4.1	Sampling: as per part 2.1	$	93,000
4.2	Analyses:		
	a) Benthos: sorting, processing, identification to species level: average $500 / sample X 96 (range $200 - $800)		48,000
	b) Grain Size: 96 X $60		5,760
	c) TOC: 96 X $30		2,880
	d) Trace Metals in Sediment: 80 samples X $60		4,800
	e) Trace Metals in Tissue: 32 samples X $60		1,920
	f) n-alkanes, PAH in sediment: 80 X $390		31,200
	g) PAH in tissue: 32 X $300		9,600
	h) Quality Assurance (field and laboratory): (add an extra 15% of costs d - g)		7,000
	TOTAL SAMPLING AND ANALYSIS	$	199,160

The cost estimate indicates that all sampling and analyses can be completed within budget: allowing approximately 10% for reporting: (≈ $20,000) the total budget would be under $225,000, well within the budgetary constraints.

It would be possible, therefore to suggest sampling additional sites or alternately; to allow for an additional sampling trip in winter if all sampling could not be completed in one trip.

If the estimated cost had been over budget, then cuts would be required. This could be done by reducing the number of analyses. It would be preferable to collect as many samples as possible since it would be very expensive to collect additional samples but relatively inexpensive to add additional analyses if that were required.

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