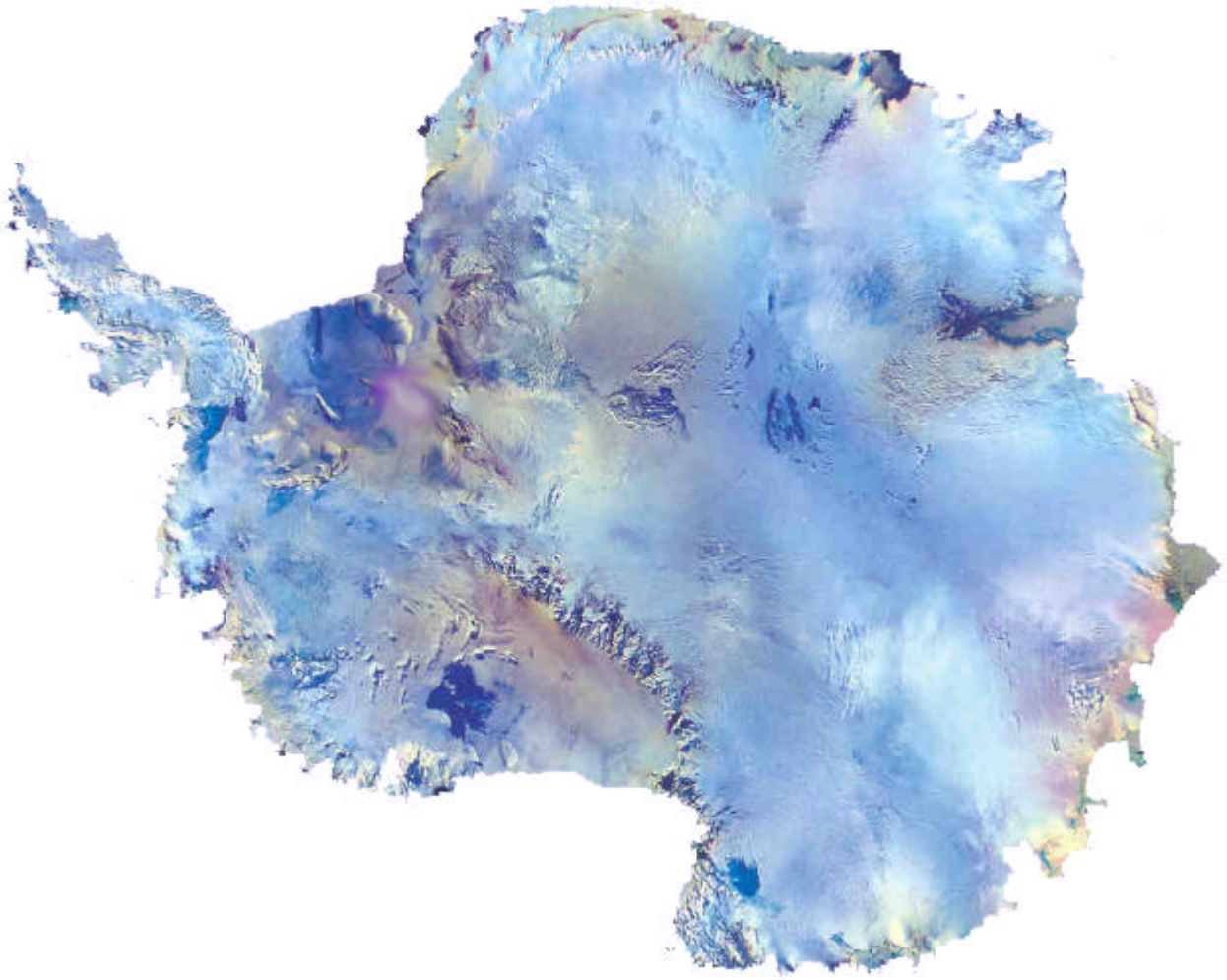
Antarctic Environmental Monitoring Handbook



Council of Managers of National Antarctic Programs

Scientific Committee on Antarctic Research

Antarctic Environmental Monitoring Handbook

Standard techniques for monitoring in Antarctica

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FOREWORD

Introduction

The development of the Protocol on Environmental Protection to the Antarctic Treaty in 1989, and its accession by Antarctic Treaty Consultative Parties in 1991, intensified the need to implement regular environmental monitoring programs throughout Antarctica. Whilst environmental monitoring has been undertaken in Antarctica over many decades, the Protocol specifically requires regular and effective monitoring of the impacts of activities and verification of predicted impacts.

At its 1994 meeting, the Antarctic Treaty Parties requested COMNAP and SCAR to conduct technical workshops that would develop an approach to monitoring which would be scientifically sound, practical and cost effective. The outcomes of the two workshops were presented in July 1996 in a report on "Monitoring of Environmental Impacts from Science and Operations in Antarctica". The report's conclusions were endorsed by the Treaty Parties and actions subsequently initiated through COMNAP and SCAR towards implementation.

One of the key recommendations was the development of a technical handbook that would be used as a guide on the scientific protocols for environmental monitoring programs. This handbook has been developed to serve that need and represents the efforts of a large number of individuals to provide an invaluable resource for implementing monitoring programs in Antarctica.

The coordinated effort to define complementary monitoring processes bodes well for the continued protection of Antarctic resources and values and for minimising human impacts through scientifically sound management of human presence in Antarctica.

Acknowledgements

Many individuals contributed to the development of this manual. Special thanks are due to the Project Director, Dr Mahlon C Kennicutt II, who not only managed the development of the handbook but also was deeply involved in organising and participating in the COMNAP/SCAR technical workshops on environmental monitoring.

Several members of the Geochemical and Environment Research Group in the College of Geosciences A&M University aided in compiling and editing the manual. Drs Guy Denoux, Terry Wade and Jose Sericano assisted in compiling the analytical methods. Ms Dianna Alsup assembled the protocols related to terrestrial monitoring, snow analyses and station activities. Dr Gary Wolff developed the data management sections and aided in production of the manual. Dr Wendy Keeney-Kennicut reviewed the manual for content and accuracy. Ms Debbie Paul was responsible for the typing, editing and final production of the manual.

The manual was greatly improved by the reviews and comments provided by the COMNAP/SCAR Project Team which was coordinated by Ms Birgit Njastad (Norway) and included Dr Heinz Miller (Germany), Dr Jan-Gunnar Winther (Norway), Dr David Walton (UK) and Dr Joyce Jatko (USA). The manual was also circulated for review and comment to members of the Antarctic Environmental Officers Network (AEON), COMNAP and SCAR.

Ms Gillian Wratt
Chairperson
COMNAP

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List of Acronyms

AA	Auto Analyzer
AAS	Atomic Absorption Spectrophotometry
ACS	American Chemical Society
AEON	Antarctic Environmental Officers Network
AMANDA	Antarctic Muon and Neutrino Detector Array
AMAP	Arctic Monitoring and Assessment Project
APAC	Aromatic Pesticide Analyte Calibration
APHA	American Public Health Association
ASTM	American Society for Testing and Materials
BCR	Community Bureau of Reference
BEA	Bile Esculin Agar
BHI	Brain Heart Infusion broth or ag
BOD	Biological Oxidation Demand
CAP	College of American Pathologists
CCB	Continuing Calibration Blank
CCV	Continuing Calibration Verification standard
CD	Compact Disk
CLP	Contract Laboratory Program (EPA)
COC	Chain of Custody
COD	Chemical Oxidation Demand
COMNAP	Council of Managers of National Antarctic Programs
CRDL	Contract Required Detection Limit
CRM	Certified Reference Materials
CV	Coefficient of Variation
CVAAS	Cold Vapor Atomic Absorption Spectroscopy
DBMS	Data Base Management System
DEM	Digital Elevation Model
DI or DIW	Deionized Water
DO	Dissolved Oxygen
DUP	Sample Duplicate
ECG	Environmental Coordinating Group
EDL	Electrodeless Discharge Lamp
EMSL	Environmental Monitoring Systems Laboratory
EPA	Environmental Protection Agency
EPICA	European Project for Ice Coring in Antarctica
FAAS	Flame Atomic Absorption Spectrophotometry
FAS	Ferrous Ammonium Sulfate
FI	Fluorescence Intensity
FID	Flame Ionization Detector
GBW	National Research Center for Certified Reference Materials
GC	Gas Chromatograph
GC/MS	Gas Chromatograph/Mass Spectrometer
GFAAS	Graphite Furnace Atomic Absorption Spectrophotometry
GIS	Geographic Information System
GISP	Greenland Ice Sheet Project
GPS	Global Positioning System
HCL	Hollow Cathode Lamps
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography

ICB	Initial Calibration Blank
ICP	Inductively Coupled Plasma
ICSA	Inter-element and background correction factors for trace metals analysis on the ICP
ICS	Interference Check Sample
ICV	Initial Calibration Verification
IDL	Instrument Detection Limits
IGS	International Glaciological Society
IPAR	Initial Precision and Accuracy
IRMM-BCR	Institute for Reference Materials and Measurements-Community Bureau of Reference
IR	Infrared Radiation
IS	Instrumental Spike
ISE	Ion Selective Electrode
I.S. Reference	Internal Standard Reference
LBS	Laboratory Blank Spike
LCS	Laboratory Control Sample
LDPE	Low Density Polyethylene
LTL	Lower Threshold Limit
MDL	Method Detection Limit
mEI	Basal Medium Agar
MF	Membrane Filter
MICRO	Glass Cleaning Solution
ML	Minimum Levels
MS	Matrix Spike
MSD	Matrix Spike Duplicate
MSDS	Material Safety Data Sheets
NA	Not Applicable
NBS	National Bureau of Standards
NCCLS	National Committee for Clinical Laboratory Standards
NIST	National Institute of Standards and Technology
NOAA	National Oceanic and Atmospheric Administration
NOS	National Ocean Service
NRCC	National Research Council of Canada
NS&T	National Status and Trends Program
ORCA	Ocean Resources Conservation and Assessment
OSI	Ocean Scientific Internaitonal Ltd.
PAD	Phenylarsine Oxide
PAH	Polycyclic Aromatic Hydrocarbons
PB	Preparation Blank
PCB	Polychlorinated Biphenyls
PE	Performance Evaluation
PFTBA	Perfluorotributylamine
PTFE	Polytetrafluoroethylene
PI	Principal Investigator
PICO	Polar Ice Coring Office of the National Institute of Polar Research
PVC	Polyvinylchloride
QA	Quality Assurance
QAMP	Quality Assurance Management Plan
QAPP	Quality Assurance Project Plan
QC	Quality Control
RF	Response Factor

RM	Reference Material
RPD	Relative Percent Difference
RRF	Relative Response Factors
RSD	Relative Standard Deviation
RT	Retention Time
SCAR	Scientific Committee on Antarctic Research
SCOR	Scientific Committee on Oceanic Research
SCRC	Sagami Chemical Research Center
SIM	Selected Ion Monitoring
SOPs	Standard Operating Procedures
SOW	Substitute Ocean Water
SPU	Specific Plant Unit
SRM	Standard Reference Material
SWE	Snow Water Equivalence
T	Temperature
TC	Total Carbon
TCLP	Toxicity Characteristic Leaching Procedure
TFE	Teflon
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
TPH	Total Petroleum Hydrocarbons
TSS	Total Suspended Solids
UNEP	United Nations Environment Program
UNESCO	United Nations Educational, Scientific, and Cultural Organization
USEPA	United States Environmental Protection Agency
UV	Ultraviolet

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SECTION A

ADMINISTRATIVE AND PRE-ANALYSIS ASPECTS OF ENVIRONMENTAL MONITORING

CHAPTER 1.
INTRODUCTION

This handbook provides guidance on standard techniques and methodologies for a first tier of indicators for monitoring programs in Antarctica. The handbook is intended to encourage greater comparability of results across the wide spectrum of activities conducted under the auspices of monitoring as required by the Protocol on Environmental Protection to the Antarctic Treaty. Adoption of common techniques increases the value of monitoring activities by encouraging the collection of comparable data by all Treaty countries. Intercomparability will facilitate continent-wide interpretation of monitoring results allowing each program to benefit from others' experiences. While recommended methods are detailed, alternative methods may be appropriate as long as data of known quality is produced. While the approach is to adhere to reliable and intercomparable methodologies, any data produced under strict quality assurance guidelines will provide the needed information to managers of national Antarctic programs for sound, effective scientific-based decision making.

1.1 Purpose

In July 1996, the Scientific Committee on Antarctic Research (SCAR) and the Council of Managers of National Antarctic Programs (COMNAP) published the results of two workshops entitled "Monitoring of Environmental Impacts from Science and Operations in Antarctica" (Kennicutt et al. 1996). A wide range of issues related to monitoring in Antarctica were addressed in the report. The workshop recommended that a technical handbook of standardized methods be prepared for the key environmental indicators identified by the workshop. COMNAP subsequently developed the Terms of Reference for the work (Table 1.1). The following handbook was produced in response to this mandate and its content has been reviewed by a Project Team consisting of two members of the Antarctic Environmental Officers Network (AEON) of COMNAP, the chair of the COMNAP Environmental Coordinating Group (ECG), and a SCAR representative.

The intent of this handbook is to provide guidance for the measurement of a first tier of indicators that are most relevant to monitoring impacts due to scientific stations and the associated support activities. The mandate is also to provide methods that are relatively simple and cost-effective given the limited resources that most national Antarctic programs have for monitoring activities. Within this context, it is also important that monitoring provide relevant and unambiguous information to support sound management decisions at Antarctic stations.

In most instances, well-tested and proven techniques and methodologies that are routinely used in monitoring programs world-wide were selected for inclusion in the handbook. As monitoring efforts develop in Antarctica, more definitive methods may be needed to answer the more complex issues faced by managers of national Antarctic programs. In many cases, an assessment of the current status of the system can be made utilizing the techniques detailed in this handbook. The data generated can be used to indicate whether more extensive investigation or additional directed monitoring is needed. The methods proposed provide a first order estimation of the status and changes in the quality of the environment at scientific stations.

In the coming years as data is collected and experience is gained, it will be important to review and revise the suggested methods to ensure they adequately meet the aims of monitoring. Advances in technologies and methodologies should also be adopted as techniques are improved. Other acceptable methods, that give comparable information, may also be added to the current compendium of methods as appropriate. In addition, as baseline information is collected it may become apparent that other portions of the system or other influencing factors or agents need to be measured to more fully understand and interpret monitoring results. As mentioned, more complex questions directly related to deterioration or change in the more visible resources and values of the Antarctic continent, in particular the wildlife, may warrant the adoption of methods more directly indicative of biological effects. Biology based measurements will need to be carefully considered in light of the magnitude of natural variability in biological communities in Antarctica and the long timeframes over which change occurs. However, certain biology-based measurements such as change in community structure, biological uptake of contaminants, production of metabolites, and more subtle indicators of stress in organisms and communities may be appropriate indicators for monitoring programs. While an ideal monitoring program would include direct measurement of the relevant attributes of biological resources, many challenges still remain in perfecting biology-based indicators. Research is needed to develop a better understanding of the time frames of biological changes, the extent and nature of natural variability in important populations, and the linkages between biological responses and causative agents. Until these complex questions can be resolved, direct monitoring of biological resources will be of limited use in developing management strategies in Antarctica.

This manual should be updated and amended on an as needed basis. The methods can be revised or replaced when sufficient information is available to justify a revision. This manual is intended to encourage synergy amongst the ever-growing number of monitoring programs in Antarctica by providing for the collection of comparable data.

1.2 Handbook Preparation

In preparing this handbook, a variety of sources of information were consulted (Table 1.2). Scientific protocols already being used were the “methods of choice” including those proven useful in monitoring programs world-wide. Methods of demonstrated accuracy and precision through testing and intercalibration were preferred. The methods of the Arctic Monitoring and Assessment Project (AMAP) were of special interest. However, AMAP methods are targeted for regions that contain significant human populations and have relatively high levels of contamination that are not expected to be encountered in Antarctica. The information compiled by COMNAP's “Summary of Environmental Activities in Antarctica, 1998” was used to evaluate practices that are already in place and their performance to date. Contributors to the COMNAP summary and others were canvassed to develop further information related to the methods used and to incorporate the experiences gained from the application of monitoring methods in Antarctica. The conclusions from all relevant COMNAP and SCAR workshops were taken into account with special attention paid to the 1996 workshop report (Kennicutt et al. 1996).

1.3 Choice of Indicators

This handbook describes generally accepted standard methodologies for field collection, storage, and analysis of various matrices for the list of indicators specified below. The indicators focus on physical/chemical measurements in freshwater, seawater, wastewater, soil, marine sediments, and snow. In addition, an approach to quantify and record the type, areal extent, and level of activities that occur at scientific stations is provided as well. The indicators for which methods are provided include:

Freshwater/Seawater - suspended solids, BOD, COD, DO, pH, conductivity, nutrients, temperature, coliform bacteria, and phytoplankton.

Wastewater - suspended solids, BOD, COD, DO, pH, conductivity, nutrients, temperature, and coliform bacteria.

Soils - TOC, TIC, Cu, Pb, Zn, Cd, Hg, total petroleum hydrocarbons, and PAH.

Sediments - TOC, TIC, Cu, Pb, Zn, Cd, Hg, total petroleum hydrocarbons, and PAH.

Snow - Cu, Pb, Zn, Cd, Hg, total petroleum hydrocarbons, and particulates.

Station Activities - wastewater production/emission, fuel consumption, waste incineration, spills, and station area.

Table 1.1 Development of a Technical Handbook of Standardized Techniques for Use in Antarctica - Terms of Reference.

Terms of Reference

1. To prepare a technical handbook of standardized monitoring methodologies for a common set of indicators for use by national Antarctic programs and other Antarctic operations, for monitoring the impact of science and operations activities in Antarctica in order to comply with the Protocol requirements for monitoring.

2. The priority for the first edition of the handbook will be methodologies for monitoring the impacts of stations in Antarctica.

3. The handbook will include:

   - standardized techniques and methodologies for monitoring the principal physical and chemical indicators identified in the SCAR/COMNAP report, 1996 ("Monitoring the Environmental Impacts from Science and Operations in Antarctica", Kennicutt et al. 1996);

   - standardized techniques and methodologies for biological monitoring based on the recommended options identified in the SCAR/COMNAP report 1996;

   - guidelines for data management related to monitoring programs.

4. In preparing the handbook, the following shall be taken into account:

   - scientific protocols which already exist for monitoring the indicators identified (including those used outside Antarctica);

   - experience gained and information available through existing Antarctic monitoring activities (refer in particular to the COMNAP document "Summary of Environmental Monitoring Activities in Antarctica", Kennicutt et al. May 1998);

   - relevant conclusions set out in the SCAR/COMNAP report 1996; and

   - mechanisms to update monitoring techniques and to extend the contents of the handbook.

Table 1.2. Compilation of Methods Reviewed During the Development of this Handbook (EPA-U.S. Environmental Protection Agency, ASTM-American Society for Testing Materials, NOAA NS&T-National Oceanic and Atmospheric Administration National Status and Trends Mussel Watch Program, APHA-American Public Health Association).

Analyte	Method	Description	Source	Matrix
Acidity	EPA 305.1	Titrimetric	EPA	water, wastewater
	EPA 305.2	Titrimetric	EPA	water
Aromatics	ASTM D5831-96	Screening for fuels	ASTM	soils
	EPA 602	Purge and Trap gas chromatographic	EPA	surface, sea, wastewaters, soils, sediments, sludges
(PAH)	EPA 550, 550.1	Liquid-Liquid extraction with HPLC, Coupled Ultraviolet and Fluorescence Detection	EPA	water
	EPA 3540, NOS ORCA 130	Soxhlet extraction with dichloromethane	EPA, NOAA NS&T	soils, sediments
	NOS ORCA 130	Extraction with dichloromethane with tissumizer; Kuderna-Danish technique	NOAA NS&T	Tissues
PCB, PAH, TPH	Mudroch et al. (1997)	Recommended extraction procedures		sediment
Hydrocarbons	EPA 5030, 5030a, 5030b	Purge and Trap	EPA	water, wastewater
	EPA 8015, 8015a, 8015b	Gas Chromatography	EPA	water, wastewater
	NOS ORCA 130	Gas Chromatography/Mass Spectrometry--selected ion monitoring	NOAA NS&T	water, sediments, tissue
(Volatiles)	ASTM D4547-91	Sampling for volatile organics	ASTM	soils, sediments, wastes
BOD	EPA 405.1	Incubation, Probe	EPA	water
Characterization	ASTM D2488-93	Visual identification and description	ASTM	soils
COD	EPA 410.1	Titrimetric	EPA	water
	EPA 410.2	Titrimetric	EPA	saline waters
	EPA 410.3	Titrimetric	EPA	water
	EPA 410.4	Titrimetric	EPA	ground, surface waters
	APHA 5220b	Open Reflux method	APHA	water, wastewater
	APHA 5220c, d	Closed Reflux method	APHA	water, wastewater
DO	360.1	Probe	EPA	water--outfalls, streams
	360.1	Titration, Probe	EPA	water, wastewater
Cadmium (Cd)	EPA 213.2 CLP	Atomic absorption, furnace technique	EPA	water, wastewater

Table 1.2. (Cont.)

Analyte	Method	Description	Source	Matrix
Coliform	EPA 1600	Membrane Filter Test	EPA	water, wastewater
Copper (Cu)	EPA 220.2 CLP	Atomic absorption, furnace technique	EPA	
Decontamination Procedures	ASTM D5088	Field equipment (nonradioactive sites)	ASTM	soils, sediments, sludges, wastes
Dry Weight	NOS ORCA 130	Dry weight	NOAA NS&T	sediments
	NOS ORCA 130	Dry weight	NOAA NS&T	Tissues
Grain size	ASTM D2217-85	Wet preparation, particle size analysis	ASTM	soils
	ASTM D2217-85	Dry preparation, particle size analysis	ASTM	soils
	NOS ORCA 130	Pipette Method	NOAA NS&T	sediment
Lead (Pb)	ASTM E 1727-95	Atomic spectrometry	ASTM	soils
	EPA 239.2 CLP	Atomic absorption, furnace technique	EPA	
Mercury (Hg)	EPA 245.1	Cold Vapor Atomic Absorption Spectrometry	EPA	ground, surface, wastewaters
	EPA 245.3	Inorganic Hg detection using HPLC with ECD	EPA	ground, surface, wastewaters
	EPA 245.5	Cold Vapor Atomic Absorption Spectrometry	EPA	Soils, sediments, bottom deposits, sludge
	EPA 245.6	Cold Vapor Atomic Absorption Spectrometry	EPA	Tissues
Metals and Trace Elements	EPA 200.7, Mudroch et al. (1997)	Inductive Coupled Plasma - Atomic Emission	EPA	water, wastewaters, solid wastes
	Mudroch et al. (1997)	Inductive Coupled Plasma - Mass Spectrometry		sediment
	Mudroch et al. (1997)	Decomposition Technique		sediment
	Mudroch et al. (1997)	Flame Atomic Absorption Spectrometry		sediment

Table 1.2. (Cont.)

Analyte	Method	Description	Source	Matrix
Metals and Trace Elements	Mudroch et al. (1997)	Quartz Tube Atomic Absorption Spectrometry		sediment
	Mudroch et al. (1997)	Graphite Furnace Atomic Absorption Spectrometry		sediment
	Mudroch et al. (1997)	Slurry Atomic Absorption Spectrometry		sediment
Nitrate (NO_3)	EPA 352.1	Colorimetric, Brucine	EPA	surface, sea, wastewaters
	EPA 353.1	Colorimetric, Automated, Hydrazine Reduction	EPA	surface, sea, wastewaters
	EPA 353.2	Automated Colorimetry	EPA	surface, sea, wastewaters
C and P also	Mudroch et al. (1997)	Dry Combustion, Filter, Kjeldahl etc.		sediment
Nitrite	EPA 353.1	Colorimetric, Automated, Hydrazine Reduction	EPA	surface, sea, wastewaters
	EPA 353.2	Automated Colorimetry	EPA	surface, sea, wastewaters
C and P also	Mudroch et al. (1997)	Dry Combustion, Filter, Kjeldahl etc.		sediment
pH	ASTM D 4972-95	Paper	ASTM	soils
	EPA 150.1	Electrometric	EPA	wastewaters
	EPA 9040, 9040A, 9040B	Electrometric	EPA	wastewaters
	EPA 9041, 9041A	Electrometric	EPA	wastewaters
	EPA 9045, 9045A, 9045B, 9045C	Electrometric	EPA	soils, waste, sludge
	Mudroch et al. (1997)	General information		sediment
Sample Preparation	Mudroch et al. (1997)	Collection, Storage, Analysis		sediment
Snow	Clarke and Noon (1985)	Nucleopore filter, photometer		Particulate matter (pm)
	Clarke and Noon (1988)	Collection, Nucleopore filters		Particulate matter (pm)-elemental carbon
	Chylek et al. (1987)	Collection, Quartz and Polyvinyl Membrane Filtration,		Particulate matter (pm)
	Chylek et al. (1983)	Snow Albedo Model		Snow Cover
	Stein et al. (1996)	Time domain reflectometry, freezing calorimetry technique		Snow dry density, liquid water content

Table 1.2. (Cont.)

Analyte	Method	Description	Source	Matrix
Specific Conductance	EPA 9050, 9050A	Conductivity Meter	EPA	surface, sea, wastewaters
Temperature	EPA 170.1	Thermometer, thermistor	EPA	surface, sea, wastewaters
TOC	EPA 415.2	UV Promoted Persulfate Oxidation	EPA	drinking waters
	EPA 9060	Carbon Analyzer	EPA	surface, sea, wastewaters
	APHA 5310 B	Combustion-Infrared Method	APHA	surface, sea, wastewaters
	APHA 5310 C	Oxidation Method	APHA	surface, sea, wastewaters
	APHA 5310 D	Wet-Oxidation Method	APHA	surface, sea, wastewaters
	NOS ORCA 130	Combustion-Infrared Method	NOAA NS&T	sediments
Trace Elements	EPA 200.1	Inductively Coupled Plasma Mass Spectrometry	EPA	sea, ground, surface waters, soils, sediments, and sludge
	EPA 200.2, 200.3	Sample Preparation Spectrochemical	EPA	Tissues
	EPA 200.7	Inductively Coupled Plasma Atomic Emission	EPA	surface, sea, wastewaters
	Mudroch et al. (1997)	Slurry Atomic Absorption Spectrometry		sediments

CHAPTER 2.
QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

Quality assurance (QA) involves all of the planned and systematic actions necessary to provide confidence that the work performed conforms to the monitoring program's goals. Quality assurance encompasses quality control (QC) which involves the examination of work performed in the context of the standards agreed upon for the measurements being made. Quality assurance is particularly important to Antarctic monitoring in that each country will be pursuing its own program. In order to promote synergy and cooperation, comparable data must be collected. The basis for this cooperation will be the production of data of known quality. This combining of efforts will allow national programs to benefit from the experiences of other managers of national Antarctic programs. The same activities occur at most scientific stations even though the intensity, duration, frequency, and mixture of activities varies widely. QA is also key to detecting human induced change in areas where natural variability is expected to be high. Many impacts may be subtle and long-term reliable data collection will be needed to detect and respond to changes caused by human activities.

As a first step, an atmosphere that encourages and requires adherence to QA/QC principles must be provided. Management is responsible for ensuring that adequate resources are available to implement the QA/QC system thus assuring the quality of the data produced. The formal recognition of quality goals ensures that monitoring goals are met and that those involved in monitoring are committed to providing the highest quality performance.

Monitoring programs should be conducted based on a detailed written plan. This plan can take the form of a Quality Assurance Management Plan (QAMP), Quality Assurance Project Plan (QAPP), or other planning documents. A QAMP or QAPP details the critical elements of a monitoring program (Tables 2.1 and 2.2).

The level of documentation that is needed for a specific monitoring program will depend on the complexity of the program and the resources available. A written explanation of the goals and the procedures of the program are essential for continuity and for communicating monitoring results to others. A formal QAMP or QAPP may not be necessary, but some type of documentation of the details of the program are recommended. Those program elements that are important for future reference and for others to judge the quality of the results produced are illustrated in the attached QAMP and QAPP Table of Contents (Tables 2.1 and 2.2). This is one approach and each program can decide what documentation is most appropriate to their program. These documents are also important in training new personnel and ensuring that all involved have a common understanding of the program's goals. If external contractors are utilized for any portion of the project, they should be required to produce similar quality assurance documents that are in concert with the program's goals.

2.1 Standard Operating Procedures

While this technical handbook includes sufficient details for the conduct of various methodologies and techniques, it is recommended that each program adapt the guidelines to their specific needs. It is also recommended that the analyst refer to the original methodological

references for greater detail. In order to develop procedures that are specific to each laboratory and the instrumentation available, more detailed Standard Operating Procedures (SOPs) should be developed by each monitoring program to ensure long-term intercomparability of results. Procedures should be updated on a regular basis to provide revised and improved techniques based on hands-on experience. SOPs also facilitate the transfer of knowledge when multiple analysts are involved or monitoring occurs over many years.

A procedure should be established for developing new or modifying existing Standard Operating Procedures. SOPs should use standardized formats, be subject to a review process, and provide for management approval. The need to modify SOPs may occur as a result of the introduction of new or improved field or analytical techniques or modification of project objectives or goals. A person should be designated to review, coordinate, and prepare new or revise current Standard Operating Procedures. All who use the SOPs should be encouraged to provide suggestions and observations regarding the performance of the SOP. However, alteration of the SOP requires formal review and approval to maintain comparability amongst measurements. Appropriate testing should be performed to ensure that equivalent data is produced by any revised or new SOP. Suggested formats for three types of SOPs are provided: administrative, preparation and instrumental procedures (Tables 2.3-2.5).

One important component of an SOP is explicit identification of any specific hazard that the field team or analyst might be exposed to, especially solvents and chemicals. This should include any specific handling requirements and precautions that should be taken. In addition, clear guidance on the disposal of any hazardous waste should also be included.

Any laboratories contracted to perform analyses should be requested to prepare and submit SOPs for review and approval before the initiation of any analyses.

2.2 Standard Reference Materials

Reference materials are key to Antarctic monitoring where results will be produced by many independent programs. The routine analysis of reference materials provides a benchmark or reference for comparison of data amongst laboratories. Any bias or artifact in results will be documented from the results of laboratories analyzing the same material or material having constituents of an agreed value. Reference materials are important for the internal assessment of methodological performance as well.

Standard Reference Materials(SRMs) are used by laboratories to validate their analytical methods and assess accuracy. If Certified Reference Materials (CRMs) with known concentrations are not available, reference materials with information values can be substituted. The term standard reference material (SRM) is used to indicate either CRMs or other RMs. After validation, methods are monitored to verify that they continue to produce acceptable data and meet quality assurance objectives. The SRM matrix and analyte concentrations used should be a reasonable match to that of the samples being analyzed. It may be difficult to find SRMs that meet all of the matrix and concentration requirements, therefore compromises are inevitable. However, the availability of SRMs has greatly increased in recent years and their use is the best available approach for the determination of precision and accuracy (Tables 2.6-2.8).

SRMs have undergone extensive testing to ensure a homogeneous sample for replicate analyses to determine the precision and accuracy of a method. To calculate a standard deviation when validating a method, the minimum number of analyses is three. The resulting mean and standard deviation can then be compared to the certified or information values provided with the SRM.

The SRM is analyzed as if it were a sample using the standard analytical protocols. It is desirable to analyze the SRM as a blind test (the analyst is unaware that the sample is an SRM) to ensure that it receives no special handling or treatment. Acceptable limits should be set for SRM results based on the program's goals. The measurements may have an adequate precision (an acceptable standard deviation), but the results do not agree with the reported concentration. This is caused by a bias in the method. The method should be improved to eliminate the bias if possible or an alternative method should be chosen.

If the analyses of the SRM shows the method to have acceptable accuracy for the goals of the monitoring, then analyses of actual samples can proceed. The RM should be reanalyzed frequently to confirm that the method performance meets the QC criteria. Whenever the SRM analysis shows that the desired method accuracy is not being met, the affected sample sets are reanalyzed. It is also necessary to determine the cause of the failure and correct it. If the frequent use of SRMs is cost prohibitive, a secondary laboratory reference material, produced within the laboratory, can be used after standardization against the SRM. Like SRMs, this secondary material should be homogeneous to provide consistent results. A summary of relevant SRMs, and their sources, are provided in Tables 2.6-2.9.

If a contract laboratory is used, the frequency of analysis of SRMs should be stipulated. It is also a good practice for the organization submitting samples for analysis to intersperse SRMs as blind checks on performance.

An additional check on interlaboratory comparability is the provision of either a common sample for analysis by all laboratories ("round-robin intercomparisons") or the exchange of samples between laboratories. These types of exercises often require independent organization of the exercise to assure anonymity of the participants and full disclosure of the results. There are a range of organizations that support and facilitate these independent intercomparisons for a wide range of analytes. Participation in intercalibrations should be encouraged. In some instances, a subset of samples (i.e., 10%) is sent to a second laboratory to serve as a QA/QC check and to confirm of the quality of the data being produced.

Table 2.1. An Example of the Table of Contents for a Quality Assurance Management Plan (QAMP).

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Table 2.2. An Example of the Table of Contents for a Quality Assurance Project Plan (QAPP) for an Individual Project.

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Table 2.3. Format for an Administrative Standard Operating Procedure (SOP).

The following format is for an Administrative SOP and presents an outline for the general requirements for repetitive administrative or documentation activities. This outline is a suggested format, although the wording should be modified to reflect the content and applicability of each Administrative SOP.

1.0 PURPOSE

1.1 Summary

This procedure [establishes the selection and training requirements] *for personnel involved in the operation, maintenance, and technical support of the monitoring programs.*

1.2 Application

The provisions of this SOP apply to all operations, staff, and management.

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer. [This is a U.S. requirement.]

3.0 RESPONSIBILITIES AND AUTHORITIES

4.0 PERSONNEL SELECTION

5.0 TRAINING

5.1 Requirements

5.2 Primary Training

5.3 Supplemental Training

5.4 Continuing Training

6.0 Record Requirements

Table 2.4. Format for a Preparation Standard Operating Procedure (SOP).

A preparation SOP provides detailed procedures for field activities, describing the collection and handling of samples prior to analysis, and also delineates the steps required for the preparation of a sample for the appropriate analytical or instrumental activity. The procedures described in these SOPs ensure the sample's representativeness, non-contamination, and appropriate handling and storage.

The outline below provides suggested elements for this type of SOP. When appropriate, sections may be added, omitted, or modified, or the abbreviation "NA" (not applicable) may be used.

1.0 PURPOSE

This document provides the procedures (sample collection, field analysis, sample extraction, sample extract purification, etc.) *that are to be used in monitoring programs.*

1.1 SUMMARY OF METHOD

[This section provides a BRIEF summary of the preparation activity. This section can concisely reference other SOPs (by topic) which must be used in addition to the procedure described in this SOP. A more detailed description of such additional SOPs, including any required variances can be included later in this document.]

1.2 APPLICABILITY

1.2.1 Matrix

[This section specifies general matrices in sentence format such as biological tissue, soil/sediment, water, or may be highly specific, such as "lichens only" or may be NA for certain field procedure such as launching and recovery of sensors.]

1.2.2 Interferences

[Typically this section may state either NA or the statements similar to the following which are relevant to sample preparation:

"High purity reagents and solvents must be used, and all equipment and glassware must be scrupulously cleaned. Laboratory method blanks must be prepared for analysis to demonstrate the lack of contamination during preparation activities that would interfere with the measurement of the target analytes. Gloves, certain plastic components, and certain greases must not be used if these will result in phthalate contamination."

"Column or gel permeation chromatographic procedures are used to remove undesired co-extracted interfering components; these procedures must be performed carefully to minimize loss of target analytes. Gloves, other plastic components, and certain greases must not be used if these will result in phthalate contamination."]

"Trip or field blanks may be a specified QA/QC component. This section should also specify any protocols for handling and disposal of hazardous waste materals."

1.2.3 Special Precautions

[This section may omitted or may specify precautions which are pertinent to the preparation activity. It may refer to "Normal laboratory (or field) safety procedure...", or may contain special information from the MSDS for specific hazards or information regarding areas such as a "Caution: open samples in a biological safety cabinet"; or it may provide directions such as "Sample upstream of diesel fumes".]

1.2.4 Reporting Units

[When the Preparation SOP provides reportable data for a project, this section should specify the required reporting units, on both matrix specific and wet weight versus dry weight basis. This section may also be omitted or an NA is used when appropriate.]

1.2.5 Method Detection Limits (MDL)

[If applicable, use of tabular MDL information on a separate sheet will aid in future updates for the SOP. This section can refer to the MDL information in the Instrumental SOP.]

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer. [A U.S. requirement.]

3.0 QUALITY CONTROL REQUIREMENTS

[This section typically provides a numbered list with a definition and summary description of standard QC (i.e., preparation of a method blank; preparation of the MS/MSD per 20 samples or less, etc.) and includes applicable QC acceptance criteria for the preparation activity. QC criteria typically would include RPD calculations and any calibration requirements. Note that this section should indicate that QC criteria for analytical results are addressed in the Instrumental SOP for the method blank, surrogates, MS/MSD, duplicates or SRM/LCS.]

[This section DOES NOT describe the preparation of the QC samples or material; such information is to be included in subsequent sections of the SOP.]

4.0 APPARATUS AND MATERIALS

4.1 Glassware and Hardware

[This section provides a numbered list (i.e., 4.1.1, 4.1.2) specifying the quantities and sizes of all equipment, including sources of specialty or trademark items. The phrase “or equivalent” should follow specialty glassware or equipment when applicable.]

4.2 Instrumentation

[This section may be omitted or may provide a numbered list with the make and model of required instruments and associated components. The phrase “or equivalent” should follow specialty equipment when applicable.]

5.0 REAGENTS AND CONSUMABLE MATERIALS

[The categories listed below are suggestions which will not apply to all Preparation SOPs.]

5.1 Reagents

[Includes DI or HPLC grade water]

5.2 Analytical Standards

5.3 Standard Reference Materials

5.4 Miscellaneous Material

[Includes filter paper, glass wool, etc.]

6.0 SOP_X, SOP_Y, etc.

[If other SOPs are required before the activity described in this SOP can be performed, they should be included here and referred to in the correct sequence by the correct SOP title. **If a variance to an SOP is required, it MUST be included here.]**

[If no SOPs are referenced, this and subsequent sections of the Preparation SOP provide the sequential detailed procedures for the preparation activity. For example, describe the preparation of reagents and column materials, then the column itself, before any description of loading an extract on the column.]

7.0 Documentation Requirements [*LAST SECTION of SOP*]

[This section specifies what documentation is required to accompany samples/data at the completion of the preparation activity or where to file original documents. For example, "attach copy of lipid benchsheet", "original chain-of-custody is filed", etc.]

Table 2.5. Format for the Instrumental Standard Operating Procedure (SOP).

The following format is for an Instrumental SOP and represents the required instrument components, operating conditions, calibration, etc., which are essential for an instrumental analytical activity. This generic format should be followed, with modifications as appropriate.

The outline contains suggested elements for this type of SOP. When appropriate, a section may be added, omitted, or the abbreviation "NA" (not applicable) may be used.

1.0 Purpose

This document provides the procedures used for the analysis of [analyte type] using [gas chromatography; colorimetric determination; etc.] to support monitoring activities.

1.1 [This section provides a BRIEF summary of the instrumental or analytical system and defines the material analyzed (extracts prepared according to SOPs; sediments; aqueous samples; etc.)].

1.2 Applicability

1.2.1 Matrix

1.3 Target Analytes and Criteria

1.3.1 Target Analyte List, Area, Response Factors, and Retention Times

[Reference to a tabular format is preferred unless the analyte list is short. This section presents data for the target analytes at a given concentration, such as a midpoint calibration standard, which has been determined by the instrumental activity described in this SOP.]

1.3.2 Other analytes having similar chemical and chromatographic characteristics may also be determined using this procedure after method validation.

1.4 Matrix Specific Detection and Reporting Limits

[Reference to a tabular format is preferred unless this is for a short analyte list.]

1.5 Applicable Concentration Range

[Usually this is the instrument calibration range without extract dilution.]

1.6 Interferences

[Specific information regarding common laboratory interferents (i.e., solvents, naphthalene, phthalates) or matrix specific contaminants (plant pigments) should be included here.]

17. Special Precautions

2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer. [A U.S. requirement.]

3.0 QUALITY CONTROL

3.1 Instrument Criteria

[e.g., vacuum level; GC/MS tune criteria, etc.]

3.2 Calibration Criteria

[e.g., within X% of true value; average RF= Z; RT shift = Y minutes, correlation coefficient = 0.9950, etc.]

3.3 Criteria for QC Samples for an Analytical Batch

[The following are some examples of QC criteria.]

3.3.1 Method Blank

3.3.1.1 Analyst discretion is used when contamination is present which does not adversely effect the overall analytical effort. When this occurs, a Sample Action Request form must be completed and approved before submitting related data to management.

3.3.1.2 If major interferences for target analytes are present or if the target compounds are present in concentrations >3 times the Method Detection Limit (MDL), the entire analytical batch must be re-extracted unless the sample(s) have concentrations >10 times those found in the blank or the affected compound is not detected in the samples.

3.3.1.3 When target analytes are present in the method blank above the MDL, analytical data for those analytes in the samples must be properly annotated.

3.3.2 Surrogate Recovery

The surrogates used in each QC and field sample must meet QC recovery acceptance criteria, with the exception of samples affected by dilutions, matrix interferences, or advisory surrogates which are not used for quantitation.

3.3.2.1 Surrogate failure requires the re-extraction of a sample, except when four or more surrogates are used. One surrogate failure is allowed in this instance and the average recovery of those surrogates meeting the QC criteria for this sample may be used for calculations. When this occurs, a Sample Action Request form must be completed and approved before submitting related data to management.

3.3.2.2 When surrogate recoveries are impacted by matrix interferences or required dilutions as a result of high concentrations of analytes, the related analyte data should be annotated as estimated.

3.3.2.3 [Surrogates and recoveries specific to the analysis and matrix should be specified.]

3.3.3 Duplicates

Duplicate analyses evaluate both the actual sample homogeneity and the overall analytical system.

3.3.1 The RPD is considered invalid and is not evaluated when results are less the 10 times the MDL.

3.3.2 The Relative Percent Difference between the original and its duplicate should not exceed the established limits.

3.3.4 Matrix Spike and Matrix Spike Duplicate (MS/MSD)

Matrix spikes are used to evaluate sample homogeneity, potential effects of the sample matrix on analyte recovery, and the overall analytical system. Percent Recovery failure and/or RPD failure alone for the MS/MSD does not necessarily require re-extraction of the entire analytical batch. MS/MSD failure is sample specific.

3.3.5 Standard Reference Material (SRM)

[Preferably, the recoveries of SRM are referred to as ±30% of the SRM range for the certified or consensus value of an analyte, with no more the 35% of the analytes exceeding this criteria. The average value of the concentration range can also be used for % recovery determinations, but use of the MS/MSD or laboratory check sample is preferred.]

3.3.6 Laboratory Check Sample

[Usually applies to EPA methods; can be replaced with Laboratory Blank Spikes.]

3.3.7 Laboratory Generated Quality Control

[Blank spikes, APAC, etc.]

3.4 Analytical Criteria for Sample

[e.g., target compounds within RT window of ± B minutes; etc.]

4.0 INSTRUMENTAL CONDITIONS

4.1 Analytical Column

5.0 DETECTOR CRITERIA

6.0 INSTRUMENT [TUNING AND/OR CALIBRATION] PROCEDURE

6.1 Tune (or Calibration) Procedure
6.2 Acceptance Criteria for tune [or calibration)

Table 2.5 (Cont.)

7.0 ANALYTICAL STANDARDS

(List of analytes, solution concentrations and amounts to be used for the surrogates, internal standards, recovery standards, matrix spikes, etc., for the procedure.)

8.0 REQUIRED SAMPLE DOCUMENTATION AND IDENTIFICATION

9.0 INJECTION PROCEDURE

10.0 INSTRUMENT MAINTENANCE

xx.0 DOCUMENTATION REQUIRED FOR ANALYTICAL RESULTS [Last SOP Section]

Table 2.6. Sources of Standard Reference Materials (SRMs).

Analyte	Matrix	Names of Standard	Source
Conductivity	Ocean water	IAPSO	OSI
Hg	Estuarine sediment	#1646	NIST
Metals	Sediments	#1646	NIST
Nitrate/Nitrite	CSK	CSK Nutrient Elements	SCRC
Nutrients	Seawater	CSK	SCRC
PAHs	Mussel tissue	#1974a; HS-3, HS-4, HS-5	NIST; NRC
Particulates	Snow	#1648	NIST
PCBs	River sediment	#1939; HS-1, HS-2	NIST; NRC
Phosphate	Fortified distilled; Ocean water	CSK	SCRC
Temperature	General Use	Monograph SP 250-23	NIST
Trace elements	Open ocean water	NASS-4	NRCC
Trace elements	Ocean water	CRM 403	BCR
Trace elements	Seawater	CRM 403	BCR
Trace elements	Near Shore Seawater	CASS-3	NRCC
Trace elements, Nutrients	Ocean water	CSK	SCRC
Trace metals	Marine and estuarine sediments	BEST-1	NRCC
Trace metals	Marine sediment	BCSS-1	NRCC
Trace metals	Nearshore waters	CASS-2	NRCC
Trace metals	Marine sediment	MESS-2	NRCC
Trace metals	River water	SLRS-1	NRCC
Trace metals	Antarctic tissue (krill)	MURST-ISS-A2	IRMM-BCR
Trace metals	Antarctic sediment	MURST-ISS-A1	IRMM-BCR

BCR	Community Bureau of Reference
IRMM-BCR	Institute for Reference Materials and Measurements-Community Bureau of Reference
NIST	National Institute of Standards and Technology (formerly the NBS)
NRCC	National Research Council of Canada (NRCC)
OSI	Ocean Scientific International Ltd.
SCRC	Sagami Chemical Research Center
GBW	National Research Centre for Certified Reference Materials

Table 2.7. Standard Reference Material Sources and Contact Information.

Name of Standard	Source	Identification	Contact Information
CRM 403	BCR	Community Bureau of Reference	Community Bureau of Reference, Commission of the European Communities, Directorate General for Science Research and Development, 200 rue de la Loi, B-1049 Brussels, BELGIUM Tel: 33 2 235 31 15, Fax: 32 2 235 80 72
MURST-ISS-A2, MURST-ISS-A1	IRMM-BCR	Institute for Reference Materials and Measurements-Community Bureau of Reference	Institute for Reference Materials and Measurements Retieseweg, B-2440 Geel, BELGIUM Tel: 32(0)14 571-272, Fax: 32(0)14 584-273 E-mail doris.florian@irmm.jrc.be Community Bureau of Reference, Commission of the European Communities, Directorate General for Science Research and Development, 200 rue de la Loi, B-1049 Brussels, BELGIUM Tel: 33 2 235 31 15, Fax: 32 2 235 80 72
1646, 1648, Monograph SP 250-23, 1974a, 1939	NIST	National Institute of Standards and Technology (formerly the NBS, National Bureau of Standards)	National Institute of Standards and Technology, Office of Standard Reference Materials, Gaithersburg, MD 20899, USA Tel: 301 975 6776, Fax: 301 948 3730
HS-1, HS-2, HS-3, HS-4, HS-5, BEST-1, BCSS-1, CASS-2, CASS-3, NASS-4 MESS-2, SLRS-1	NRCC	National Research Council of Canada (NRCC)	National Research Council of Canada, Marine Analytical Chemistry Standards Program, Division of Chemistry, Montreal Road, Ottawa, Ontario K1A OR9, CANADA Tel: 613 993 2359, Fax: 613 993 2451 E-mail: crm.iert@nrc.ca National Research Council of Canada, Marine Analytical Chemistry Standards Program, Atlantic Research Laboratory, 1411 Oxford Street, Halifax, Nova Scotia B3H 3Z1, CANADA Tel: 902 426 8280, Fax: 902 426 9413
IAPSO, LNS	OSI	Ocean Scientific International Ltd.	Ocean Scientific International Ltd., South Down House, Station Road, Petersfield, Hampshire, GU32 3ET, UNITED KINGDOM, Tel: 01730 265015, Fax: 01730 265011, E-mail: osil@soc.soton.ac.uk
CSK	SCRC	Sagami Chemical Research Center (CSK - Cooperative Study of the Kuroshio and Adjacent Regions (an IOC Coordination Group)	Sagami Chemical Research Center, 4-4-1 Nishi-Ohnuma, Sagamihara-shi, Kanagawa 229, JAPAN Tel: 81-427-42-4791, Fax: 81-427-49-7631,
H2508571	GBW	National Research Centre for Certified Reference Materials	National Research Centre for Certified Reference Materials, Office of CRMs, No. 18, Bei San Huan Dong Lu, Hepingjie, 100013 Beijing, CHINA Tel: 1 613 993 2359, Fax: 1 613 993 2451

Table 2.8 lists summaries of additional SRM providers.

Table 2.8. Summary of Standard Reference Material Providers.

American Public Health Association (APHA)
1015 Fifteenth St., NW
Washington, DC 20005 USA
sposavec@awwa.org; http://www.apha.org

For 94 years APHA has distributed standards reference materials to develop more adequate and uniform methods of water and wastewater analysis. These standard methods undergo development, validation, and collaborative testing.

American Society of Testing and Materials (ASTM)
ASTM, 100 Barr Harbor Drive
West Conshohocken, PA 19428-2959 USA
webmastr@astm.org; http://www.astm.org

ASTM develops and provides standard reference materials related to technical information and services that promote public health and safety regarding soils, sediments, and water quality.

Environmental Protection Agency (EPA)
Office of Water
Washington, DC 20460 USA
public-access@epamail.epa.gov; http://www.epa.gov

The mission of the U.S. Environmental Protection Agency is to protect human health and to safeguard the natural environment (CFR Title 40-Protection of the Environment). This includes the protection of the air, water, and land. The US EPA often works in conjunction with NIST and other organizations to create CRMs and SRMs for atmospheric matrices, soils, water, wastewater, and sediments.

National Institute of Standards and Technology (NIST)
Office of Standard Reference Materials
Gaithersburg, MD 20899 USA
Tel: 301 975 6776, Fax: 301 948 3730
srminfo@enh.nist.gov; http://www.nist.gov

For 80 years NIST has distributed reference materials under 50 broad categories ranging from engineering mechanics to cement. These reference materials have certified values determined by at least two independent analytical methods or by one definitive method. NIST also offers a variety of calibration services for such devices as thermometers.

National Research Council of Canada (NRCC)
Marine Analytical Chemistry Standards Program
Division of Chemistry
Montreal Road
Ottawa, Ontario KIA 0R9
CANADA
Tel: 613 993 2359, Fax: 613 993 2451
crm.iertOnrc.ca; http://www.nrc.ca

Table 2.8. (Cont.)

National Research Council of Canada
Marine Analytical Chemistry Standards Program
Atlantic Research Laboratory
1411 Oxford Street
Halifax, Nova Scotia B3H 3Z1
CANADA
Tel: 902 426 8280, Fax: 902 426 9413
http://www.nrc.ca

The National Research Council of Canada's (NRC) Marine Analytical Chemistry Standards Program (MACSP) provides the development, production, and distribution of reference materials that support marine materials analysis. NRC certified reference materials (CRMs) have certified values determined by at least two independent analytical methods.

National Water Research Institute (NWRI)
Canada Centre for Inland Waters
867 Lakeshore Road, P. 0. Box 5050
Burlington, Ontario L7R 4A6
CANADA
Tel: 905 336 4869, Fax: 905 336 4989
yvonne.stokker@cciw.ca; http://www.cciw.ca/nwri/nwri.html

National Water Research Institute (NWRI) develops analytical chemistry methods as well as developing and conducting quality assurance programs. As part of the QA program, NWRI has developed and prepared a series of reference materials (RMs) for water analyses, and CRMs for sediment analyses that include lake sediment CRMs for PAHs, chlorobenzenes, PCBs and Se.

National Oceanic and Atmospheric Administration (NOAA)
1315 East-West, Highway SSMC3
Silver Spring, Maryland 20910 USA
sab@www.sab.noaa.gov; http://www.noaa.gov

NOAA has developed and compiled a series of reference materials for analyzing the quality of marine water and sediments. Specific SRMs relate tp water quality, sediment quality, nutrients, metals, inorganics, organics, pesticides, PCBs, PAHs, tissue analysis, as well as QA/QC procedures.

U S Geological Survey (USGS)
National Spatial Data Infrastructure (NSDI)
FGDC, USGS, 590 National Center,
Reston, VA 20192 USA
cgroat@usgs.gov; http://www.fgdc.gov

The USGS has created a consistent means to share geographic data among users by producing guidelines for data infrastructure. The National Spatial Data Infrastructure (NSDI) is defined as "the technologies, policies, and people necessary to promote sharing of geospatial data throughout all levels of government, the private and non-profit sectors, and the academic community" (USGS, 1999). This initiative meets the Federal Geographic Data Committee (FGDC) guidelines that coordinate the development of the National Spatial Data Infrastructure (NSDI).

Table 2.9. Website Listing for Methods and Other References

Analytical Chemistry Reference	http://www.anachem.umu.se/jumpstation.htm
American Public Health Association (APHA) Publications List	http://www.apha.org/media/index.htm
Arctic Monitoring and Assessment Program (AMAP)	http://www.grida.no/prog/polar/amap/
ASTM	http://www.astm.org/
British Antarctic Survey	http://www.nerc-bas.ac.uk/
CREEL Virtual Library	http://www.crrel.usace.army.mil/library/crrel_library.html
EPA 1600	http://www.epa.gov/OST/beaches/entero.html
EPA CLP Statement of Work	http://www.epa.gov/oerrpage/superfund/programs/clp/methods.htm
EPA Method 3540c	http://www.epa.gov/epaoswer/hazwaste/test/3540c.pdf
EPA Method 3611b	http://www.epa.gov/epaoswer/hazwaste/test/3611b.pdf
EPA Method 5030b	http://www.epa.gov/epaoswer/hazwaste/test/5030b.pdf
EPA Method 550.1	http://www.horizontechinc.com/Documents/notes.htm
EPA Method 8015b	http://www.epa.gov/epaoswer/hazwaste/test/8015b.pdf
EPA Method 9040b	http://www.epa.gov/epaoswer/hazwaste/test/9040b.pdf
EPA Method 9041b	http://www.epa.gov/epaoswer/hazwaste/test/9041a.pdf
EPA Method 9045b	http://www.epa.gov/epaoswer/hazwaste/test/9045c.pdf
EPA Method 9050b	http://www.epa.gov/epaoswer/hazwaste/test/9050a.pdf
EPA Method 9060b	http://www.epa.gov/epaoswer/hazwaste/test/9060.pdf
EPA Methods Index	http://www.neis.com/epaindex.html
EPA Search Engine	http://search.epa.gov/
EPA SW-846 methods	http://www.epa.gov/epaoswer/hazwaste/test
General Chemistry Reference	http://chemistry.miningco.com/education/chemistry/mbody.htm?terms=chemistry&
IASOS Antarctic Information Resources	http://www.antcrc.utas.edu.au/iasos/Resources.html
I-CHEM Environmental Sample Guide	http://i-chem.nalgenunc.com/scripts/nalge/i-chem/select/sample.asp
IRMM Certification Reports	http://www.irmm.jrc.be/rm/cert-reports.html
Italian Antarctic Research Program	http://www.pnra.it/index_inglese.html
MURST-ISS-A1	http://www.irmm.jrc.be/rm/murst-iss-a1.pdf
MURST-ISS-A2	http://www.irmm.jrc.be/rm/murst-iss-a2.pdf
National Environmental Publications Internet Site (NEPIS)	http://www.epa.gov/cincl/
National Ocean Service Search Engine	http://search.nos.noaa.gov/compass
National Oceanic and Atmospheric Administration	http://www.noaa.gov/
NIST Standard Reference Materials	http://ts.nist.gov/ts/htdocs/230/232/232.htm
Office of Ground Water and Drinking Water	http://www.epa.gov/OGWDW/
Office of Ground Water and Drinking Water Search	http://www.epa.gov/watrhome/search.html
OSHA methods	http://www.osha-slc.gov/dts/sltc/methods/index.html
SRM Index list	http://www.epa.gov/owowwtr1/info/PubList/monitoring/html/023.html
SRM Index list	http://www.epa.gov:80/owowwtr1/info/PubList/monitoring/docs/023.pdf
Standard Reference Data	http://www.nist.gov/srd
Standard Reference Materials	http://ts.nist.gov/ts/htdocs/230/232/232.htm

CHAPTER 3.
DATA MANAGEMENT

Data management plays a key role in environmental monitoring programs. An effective data management system is necessary to meet the environmental monitoring requirements of the Antarctic Treaty System. A properly designed and implemented data management system provides effective use of the information collected by environmental monitoring programs and can fulfill the information requirements of national Antarctic programs.

Data management promotes the efficient and effective use of data collected by monitoring activities. Data comparability is facilitated through the establishment of data management protocols that disseminate available data to end-users so that local data can be examined within a regional context. Easily accessible and understandable data also allows management to identify problems concerning the status and progress of sample/data collection and analysis. Thus, it provides pertinent information for the appropriate preventative or remedial measures that may need to be taken in environmental management. A properly designed data management system promotes long-term preservation of data, which is critical to the development of baseline information to assess change in the parameters measured. Data management serves as the central node for the flow of data in an environmental monitoring program.

The most fundamental objective of data management is to promote efficient dissemination and retrieval of data arising from monitoring and other related activities.

Data management is also necessary to:

(i) ensure the preservation of long-term environmental monitoring data.
(ii) facilitate the availability of and access to data.
(iii) facilitate comparisons of environmental data with adjacent monitoring programs providing a broader perspective for data interpretation; and
(iv) maximize the utility of data and consequently, the conclusions that arise from their analysis.

In essence, data management allows information collected in environmental monitoring programs to be used to make decisions in a timely and cost-effective manner. Early access to information encourages preventative rather than reactive actions. Quality control (QC) and quality assurance (QA) are fundamental components of the design and implementation of an environmental monitoring program. The high cost associated with activities in Antarctica means that questionable data waste valuable and limited resources. Poor quality or misleading data can be more problematic than an absence of data because it can be misleading and result in the diversion of resources from the real needs to false targets. To ensure proper data dissemination, data management monitors, controls, and facilitates data flow, ensuring data integrity through a step-by-step process in each phase of a program.

A data management plan would typically include: 1) data administration, 2) data control, 3) data utilization, and 4) data archiving (Figure 3.1). The integration of these elements into a data management plan is presented below.

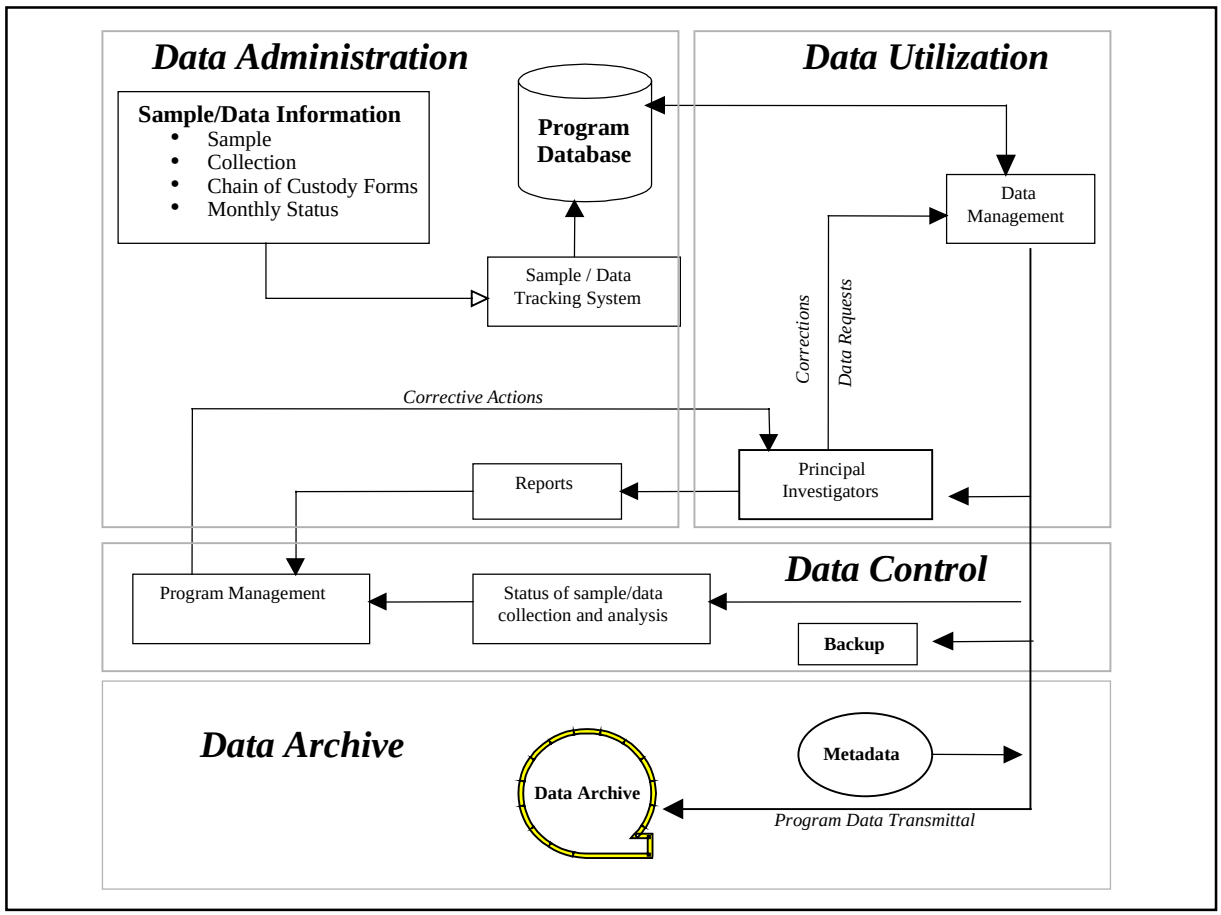


Figure 3.1. The data management plan: 1) data administration, 2) data control, 3) data utilization, and 4) data archiving.

3.1 Data Administration

The first of four elements of a data management system deals with sample and data information (Figure 3.2).

Data administration is the element of a data management plan that ensures continuous tracking and custody of samples and data. It includes documentation of data collection and possession, verification of sample collection completeness, and sample and data security through chain of custody procedures. Data administration also ensures proper formatting and recording of data in the database and distribution of data and data products as required among the investigators, report authors and agencies.

A detailed summary of the planned sample collections for the entire sampling effort should be completed prior to the field effort. With this summary a unique sample designation (sample id) for each planned sample collection element can be assigned. These sample designations can then be incorporated into a sample tracking system within the program database to ensure that the sampling effort is completed in its entirety. Uniformity in the design of sample

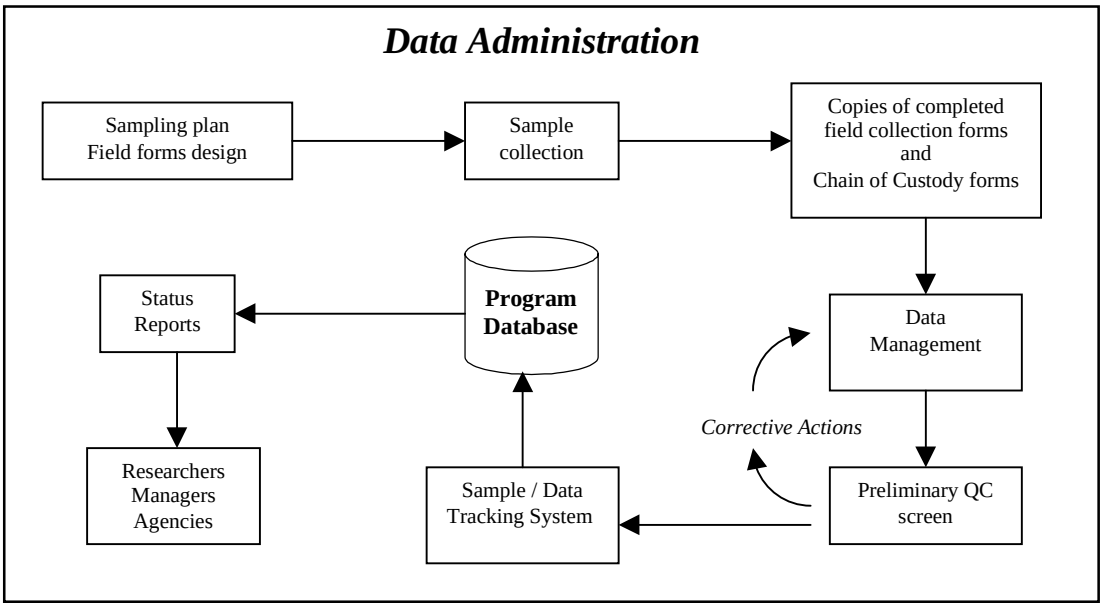


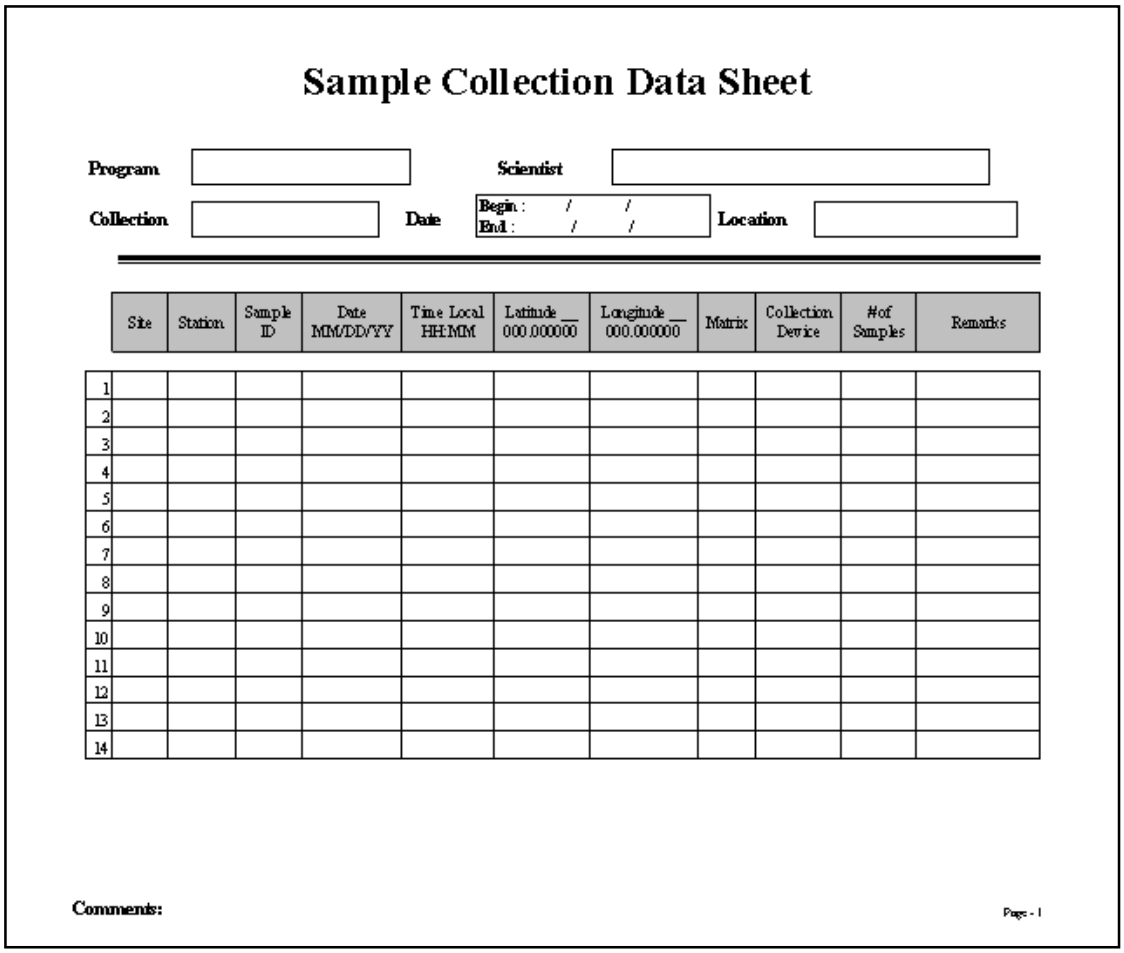
Figure 3.2. Diagram of data administration tasks.

collection forms to be used in the field facilitates subsequent documentation of data. Unique sample designators are the central component of the sample tracking system. They form the basis for documenting the collection and labeling of samples in the field. Sample designators allow for monitoring of the progress of the sampling and analysis effort in the field, through analysis to the distribution of the analytical results. An example of a field sample label that uniquely identifies the sample is illustrated in Figure 3.3. The label is placed with the sample and provides collection information in addition to a sample ID. It is best to incorporate information into the sample ID beyond simply a sequence number. In the example provided the sample ID incorporates the matrix, year, site, station and sample number.

Project: *ANT_00*	**Site:** *5D*	**Station:** *37*	**Date:** *01 / 23 / 00*
Sample ID: *S005D37-7*	**Gear:** *SCOOP*	**Matrix:** *SOIL*	**I.D.:** *D. ALSUP*
Comments: 2 cm ice/snow overlay			

Figure 3.3. Field sample collection label example.

Forms developed prior to the field effort are an integral part of the sample collection/tracking component's success. Appropriate forms allow accurate and complete documentation of the sample collection process. The uniformity gained by preparation of standardized forms facilitates documentation and ensures the completeness and accuracy of data entered into the program database. An example of a sample collection form to be used in the field is shown in Figure 3.4.



Sample Collection Data Sheet

Program _____ **Scientist** _____

Collection _____ **Date** Begin : / / End : / / **Location** _____

	Site	Station	Sample ID	Date MM/DD/YY	Time Local HH:MM	Latitude __ 000.000000	Longitude __ 000.000000	Matrix	Collection Device	#of Samples	Remarks
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											

Comments:

Page - 1

Figure 3.4. Sample collection field form example.

Careful attention to designing data collection forms promotes accurate, efficient data collection and a smooth transition from the field into the project database. In addition, thoughtful form design prevents neglecting items whose omission may compromise quality control, prevent complete statistical analyses or degrade the accuracy of any conclusions.

Collection forms are designed to ensure that all essential information is recorded in the field. In addition, the forms are designed to ensure that:

1) data are collected in the most efficient order;
2) all pertinent experimental information (sample type, location, sampling date, sampling time, etc.) is included;
3) areas on the form where observations are recorded are clearly labeled and adequate space is available to accommodate the maximum expected value, assuring simplicity and legibility of the data; and
4) additional space for recording pertinent comments is available to assure correct interpretation of the observations.

Immediately after each field effort is completed, copies of the field collection forms are forwarded to Data Management. These forms can be scanned electronically and placed into the program database for permanent documentation archival. Information contained in the copies of the field collection forms is entered into a sample/data tracking system and the status of the samples collected during the particular field effort is updated. Reports can then be produced that describe and define the sample collection information. The reports are distributed to the research team, providing an overview of data collection and a reference to sample and location nomenclature.

Continuous tracking of samples and data is accomplished using a sample/data tracking module in conjunction with tracking protocols. Custody of samples and data is documented by chain-of-custody forms and data transmittal forms for each change of possession. These forms provide the necessary security as one custodian relinquishes the samples to the next custodian with signatures. Dates and times of transfer are noted on the forms. An example of a chain of custody form is shown in Figure 3.5.

CHAIN OF CUSTODY RECORD

Project Name/No. _____ Samples Shipment Date _____
Project Manager _____ Lab Destination _____
Purchase Order No.(s) _____ Lab Contact _____
Project Contact/Phone _____ Carrier/Waybill No. _____

Program / Site Identity	Sample Number	Description / Type	Date/Time Collected	No. of Containers	Analysis	Comments/Condition on Receipt

Special Instructions:

1. Relinquished by: (Signature/Affiliation) Date: Time:
2. Relinquished by: (Signature/Affiliation) Date: Time:
3. Relinquished by: (Signature/Affiliation) Date: Time:

Comments:

Figure 3.5. Example of Chain of Custody Form.

Before a data file is incorporated as valid data into the program database, it is subjected to a data screening analysis--a quality control procedure designed to minimize errors. Sample collection data sheets or laboratory reports are compared to data file listings reviewing the latter for accuracy and completeness (Figure 3.6). This screening includes range checking and thematic scanning of data to detect potential outliers, calculation of a statistical summary, which includes minimum, maximum, and mean values, numbers of valid observations, and total number of observations in the data file. This information and tabular listings of the data file are sent to the appropriate investigator for review. The investigator reviews the printouts and

statistics for errors. Any errors in the data file are corrected, and new printouts are generated and sent to the investigator. After the investigator certifies that the data file is valid, the data file is incorporated into the program database.

Sample Collection Status

	Field Date	01-Dec-99		15-Dec-99		31-Dec-99		14-Jan-00		
		Target	Collected	Target	Collected	Target	Collected	Target	Collected	Total by Analysis
Analysis	Matrix									
PAH	TISS	55	55	48	48	20	20	19	19	142
	SED	55	55	9	9	27	27	6	6	97
	WAT	35	35	35	35	35	35	35	35	140
Trace Metal	TISS	55	55	48	48	20	20	19	19	142
	SED	55	55	9	9	27	27	6	6	97
	WAT	35	35	35	35	35	35	35	35	140
Meiofauna	SED	56	56	48	48	68	68	19	19	191
Macrofauna		56	56	48	48	20	20	19	19	143
TOC	SED	25	25	48	48	20	20	27	27	120
GrnSiz	SED	40	40	48	48	27	27	27	27	142
	Total by Field Date	467	467	376	376	299	299	212	212	1354

Figure 3.6. Example of Sample Status Form.

Copies of all chain-of-custody forms (Figure 3.5) and data transfers are sent to data management. This information can be used to update the status of the samples. It primarily consists of files of milestones for each sample. Status milestones are generally as follows:

1) sample collection in the field,
2) sample transmittal and receipt by the laboratory,
3) processing status within the laboratory and completion of the analysis,
4) transmission of the analytical results to data management,
5) data entry,
6) data validation,
7) incorporation of validated data into the program database,
8) transmittal of the sample/data to the final repository, and
9) archival.

3.2 Data Control

The purpose of data control is to monitor progress of the data flow, identifying gaps in the information supplied and to signal further processing requirements. Data control procedures enable the documentation of data availability, data reduction, and data analysis to be determined whenever necessary (Figure 3.7).

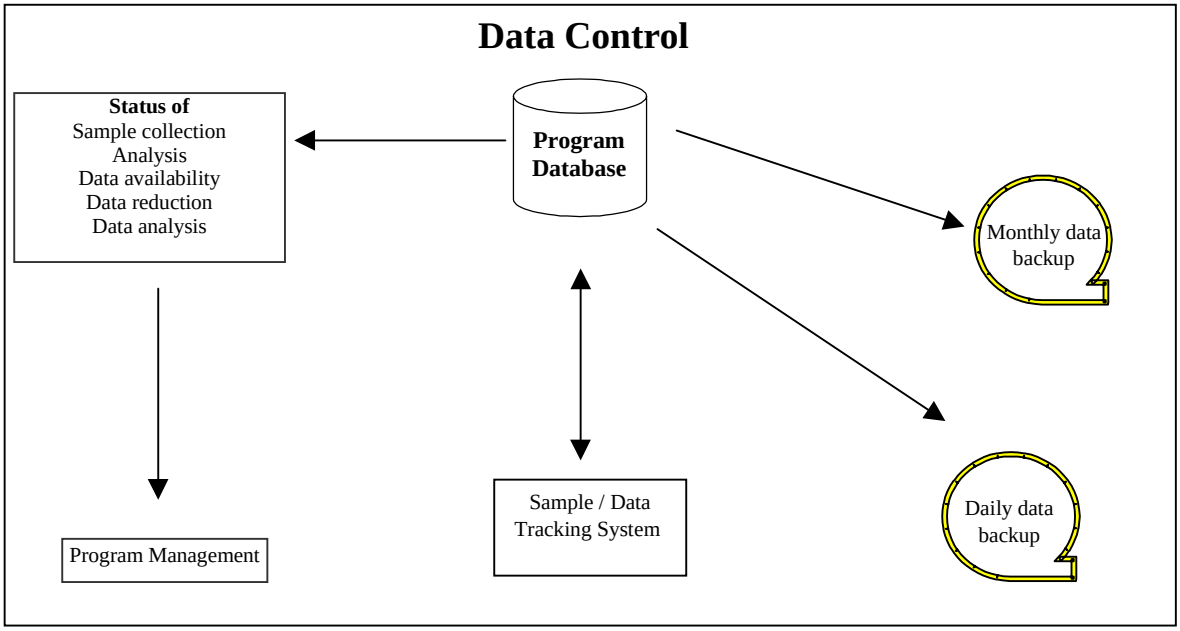


Figure 3.7. Data Control.

Data control is accomplished as part of the sample/data tracking module, the implementation of which enables data management to ascertain quickly the status and location of any given sample and to generate summaries of the status of samples and data processing. These reports enable timely decisions to be made that alleviate processing bottlenecks. Gaps in the data provided to data management will be immediately obvious by comparison of the sample/tracking files to the data received. Another element of data control is routine backup of computer files. This ensures efficient recovery of project files if a catastrophic event occurs, such as hardware failure. All files should be backed up daily. Monthly backups are performed at the end of each month and stored off-site in a controlled access and environment location.

3.3 Data Utilization

Data utilization ensures that all data are processed, validated, and made available as needed to study participants and that these data are retrievable as necessary or desirable for future analysis (Figure 3.8).

Processing of all data is assured by the sample/data tracking protocol. Quality control via data entry and validation procedures ensures that only validated data are entered into the project database. All necessary data are consequently validated and available for analysis and interpretation. Documentation of the files contained in the program database ensures that these data are retrievable for future analyses.

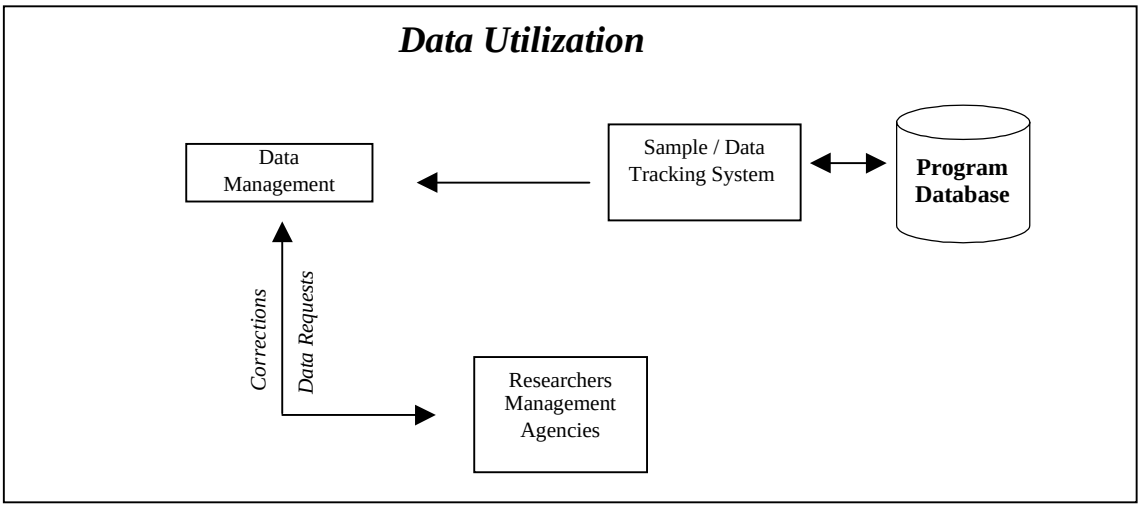


Figure 3.8. Data Utilization.

3.4 Data Archive

Data archival is a method for the safe storage and easy retrieval of all accumulated data, photographs and videotapes (Figure 3.9). Data archives should be maintained on high capacity, removable media. One backup should remain in data management's possession. Files will be routinely archived to this tape. The other backup should be maintained in a separate location. Archived files that are accumulated on the first archive tape will be periodically copied to the off-site tape. This procedure assures the permanency of the data archives.

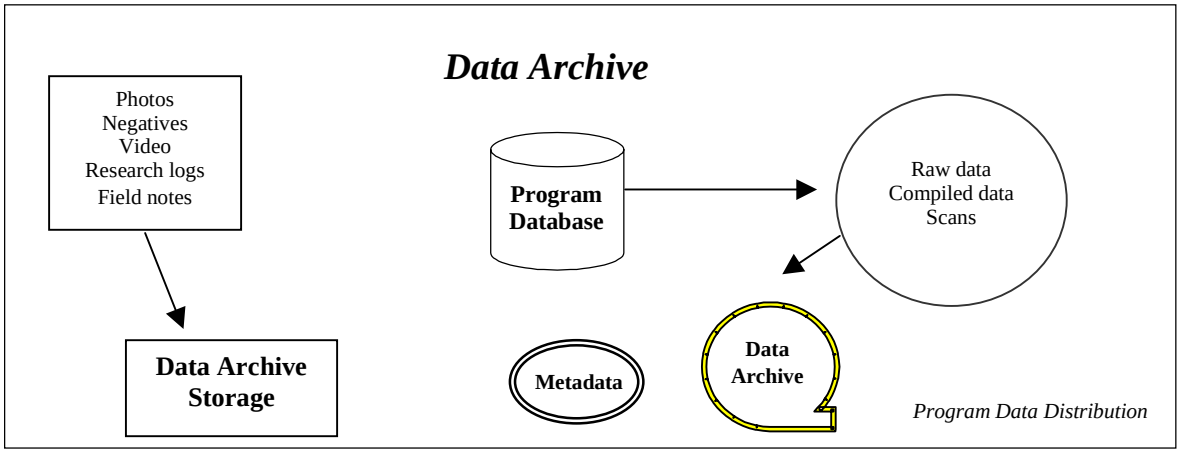


Figure 3.9. Data Archive.

Photographs can be scanned to provide a digital copy and archived on CD to provide for safe storage of the image. All original photographs and negatives are maintained in a separate, environmentally controlled storage area. Program videotapes should be copied and stored in a similar fashion.

All data collected during the course of a project should be accompanied by metadata that completely describes the physical and logical sizes and formats of all files in the data set. Also, it should provide sufficient information and references to completely identify all of the data in the data sets.

All data should be associated with "metadata" or data about the data, i.e., information describing the data in question. Amongst other information, this will provide details of the investigators or data source, collection, content, range and resolution of the data and quality assurance information. This metadata should be intimately linked to the data so that it is impossible to access a dataset without also receiving information about that dataset. Thus data of different origins (e.g., data collected as part of scientific research or as part of a monitoring program) and quality will be easily identifiable. A metadata file should be associated with all data and should conform to the guidelines as established by the National Data Centers as part of the Antarctic Data Directory.

3.5 Geographic Information Systems

A geographic information system (GIS) is a computer-based tool for viewing, mapping and analyzing phenomena that are spatially situated (Clarke 1997). GIS technology integrates common database operations with functions and tools, such as data storage, analysis, and display of geographic information as well as spatial data integration with other data resources. Geo-referenced data contain an explicit geographic reference, such as a latitude and longitude or national grid coordinate, or an implicit reference such as objects tacitly related to each other in space (i.e., distance) so that thematic data layers can be linked geographically (Figure 3.10). Geographic information systems view two fundamentally different types of geographic data-vector and raster. Vector data is encoded and stored as a collection x,y coordinates that form points, lines, and polygons. The location of a point feature, such as a sampling point, can be described by a single x,y coordinate. Linear features, such as roads and rivers, can be stored as a collection of point coordinates. Polygonal features, such as buildings or river catchments, can be stored as a closed loop of coordinates with an area and perimeter. Vector data are useful for describing discrete features, but less useful for describing continuously varying features such as soil type. Raster data models continuous features as an image comprised of a collection of grid cells, for example a scanned map or picture. Both vector and raster data are useful for storing geographic data and both have unique advantages and disadvantages. Once digitized, data types required for a particular GIS project will have to be transformed to the coordinates and scale necessary to view and analyze phenomena of interest on a landscape. Elements of geographic information are discernable at a variety of scales as reported by Kennicutt and Wolff (1998), and before this information can be integrated, it must be transformed to the appropriate scale for analysis (dependent upon the degree of detail or accuracy required).

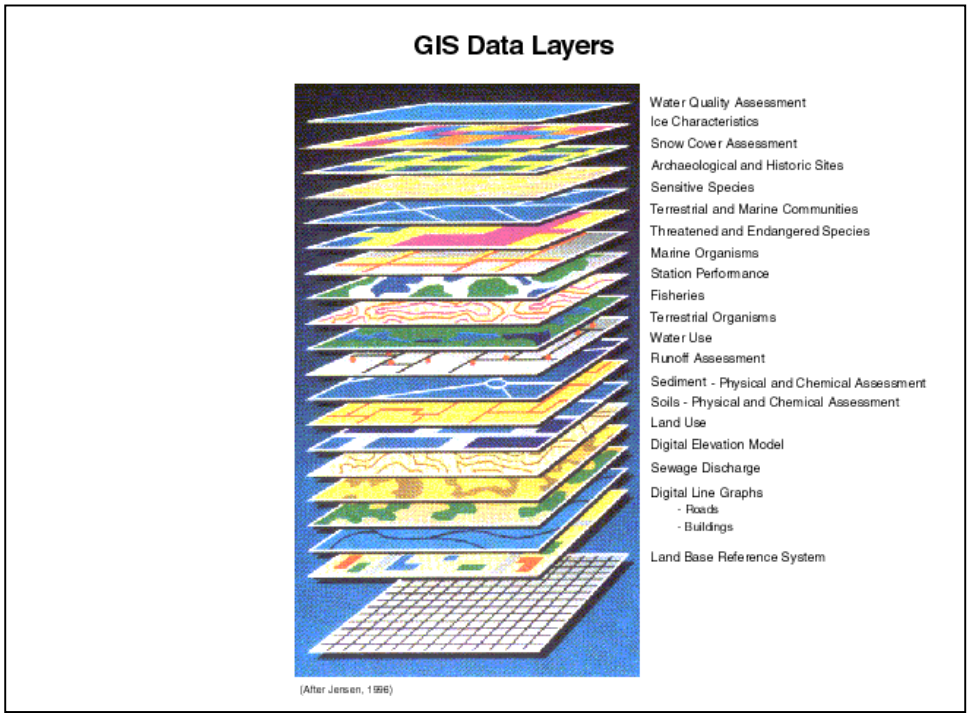


Figure 3.10. An example GIS composed of data layers of spatial information registered to a common map projection.

CHAPTER 4.
SAMPLE COLLECTION, HANDLING, AND PRESERVATION

Proper sample collection, handling, and preservation is critical to any monitoring program. The quality of data produced is dependent on collecting a representative sample whose contents are preserved intact until the time of analysis.

Sampling in support of Antarctic monitoring programs presents special challenges in that the weather is often severe and sample collection may require the sampling of or through a frozen medium. While it is beyond the scope of this manual, a brief discussion of the types of techniques available for "through the ice" sampling is provided. Special circumstances encountered include collection of samples under the ice, keeping sampling access holes open, freezing or preserving of samples upon retrieval, and performing these difficult operations in a clean non-contaminating manner.

It is also recognized that many Antarctic programs will not be equipped to carry out the analysis of samples themselves. The samples may be exported from the continent for analysis in commercial laboratories. In addition, all sampling locations will not be in close proximity to laboratory facilities that can perform the analyses on site. This requirement to transport samples from the site and possibly to laboratories within the home country presents special challenges for the handling and preservation of samples in a manner that maintains the integrity of the samples. Such things as sample size, sample type, and the chemicals used as fixatives may all be concerns for transported samples. While it is not possible to address all of the issues that might be encountered, this chapter provides general guidance on the procedures that will aid in coping with these unique characteristics of monitoring in Antarctica. Within each method, a brief summary is provided of any issues that one should be aware of when considering the sampling, handling and preserving of samples in Antarctica.

Fundamental to all sampling techniques is prevention of the introduction of foreign materials that will interfere with the intended analysis. In many cases this requirement is easily met by cleaning the sampling gear and receiving containers by standard methods. It is also important that foreign materials from the surrounding atmosphere be prevented from being introduced to the sample during collection activities. Often the sampling person will need to wear appropriate gloves or other coverings to prevent contamination by the collector. The requirement for "clean" conditions becomes more and more demanding as the concentration of the analytes of interest gets lower and lower. The addition of small amounts of interferants will consequently cause large mis-estimations in analyte concentrations. The types of interferants are dependent on the parameter being measured and thus cleaning techniques are discussed on a method-by-method basis. For example, plastic bottles cleaned by acid are appropriate for water samples collected for trace metal analysis but glass bottles cleaned with detergent, distilled water, and solvents are preferred for organic contaminants such as hydrocarbons. Various preservation techniques can be employed from freezing to chemical fixatives depending on the parameter of interest and the delay between collection and analysis. These preservation methods must retain the sample's original attribute of interest at the time of collection and also must not introduce interferants into the sample.

In some instances, “traveling” and “field blanks” are incoporated as part of the analytical protocol. In these instances, sample containers accompany the equipment into the field and are subjected to all of the events during sampling other than those of the actual sampling. Reagents are added and the “blank” is returned to the laboratory for analysis. Should contamination occur, “field blanks” are useful in identifying the portion of the procedure that leads to contamination and thus suggests a remedy for any future sampling. There also needs to be procedures in place to dispose of any hazardous materials (i.e., solvent rinses) generated in the field.

4.1 Wastewater

Sampling program objectives dictate the location and frequency of wastewater sampling. For example, to determine if water quality objectives are being met for a sewage treatment plant, sample locations are typically chosen at the inflow to the treatment facility, the effluent from the treatment facility, and the water supply. Representative sampling is an important consideration for properly characterizing wastewater streams. Chemical and biological levels in wastewater should be considered dynamic variable since wastewater flow rate and wastewater quality continuously change. Obtaining samples that represent the wastewater flow over time periods of days, months and years is difficult at best. Diurnal fluctuations occur in concentration and flow volume; seasonal fluctuations occur in concentration, flow volume, and temperature; and lab related contributions to the system might cause wastewater characteristics to change on a short- or long- term basis. Given the variable nature of wastewater, it is advisable that samples be collected that represent “average” characteristics and approximate characteristics under more extreme conditions. The wastewater quality would be expected to vary inversely with the number of individuals present on a station at any given time. Wastewater treatment facilities vary from discharging raw sewage to processing the sewage on-site prior to discharge depending on the station and the host country.

Wastewater samples can be collected manually or by using automated samplers. Care must be taken to avoid the introduction of extraneous materials when using either method of wastewater sampling. There is minimal initial cost for manual sampling; however, automated samplers are particularly well suited to higher sampling frequencies. Some advantages and disadvantages of manual and automated sampling are tabulated in Table 4.1 (from *EPA Handbook for Sampling and Sample Preservation of Water and Wastewater*, 1982). An automated sampler will have a sample gathering subsystem (mechanical, forced flow, or suction lift systems), a sample transport system (typically flexible plastic tubing), and a sample storage system (typically a two gallon container for a composite sampler). Wastewater samplers are commercially available that collect a time weighted composite sample. A grab sampler is an example of a manual sampling method that typically consists of a telescoping pole that accommodates various sized sample collection bottles at the distal end. Grab samples can also be collected from the wastewater stream using a pump, scoop, vacuum, or other suitable device. Grab samples are individual discrete samples collected over a period of time not to exceed 15 minutes. These samples will characterize the water quality at one particular time. Collection of a composite sample consisting of a number of discrete grab samples is necessary for determining average wastewater concentrations and for calculating mass per unit time loading. Each discrete sample should be at least 500 mL and each composite sample should be a minimum of 7.57 L. This will provide data that is considered representative of average wastewater characteristics throughout the day while minimizing sample holding times. A careful review of flow

Table 4.1. The Advantages and Disadvantages of Manual and Automatic Sampling of Wastewaters (EPA 1982).

Type	Advantages	Disadvantages
Manual	1) Low capital cost.	A) Probability of increased variability due to sample handling.
	2) Compensates for various situations.	Inconsistency in collection.
		B) High cost of labor, assuming several samples taken on a daily basis.
	3) No maintenance.	Repetitious and monotonous task for personnel.
	4) Can collect extra samples in a short time when necessary.	
Automatic	1) Consistent samples.	A) Considerable maintenance for batteries & cleaning.
	2) Probability of decreased variability caused by sample handling.	Susceptible to plugging by solids.
	3) Minimal labor requirement for sampling.	B) Restricted in size to the general specifications.
	4) Has capability to collect multiple bottle samples for visual estimate of variability & analysis of individual bottles.	Sample contamination potential

monitoring records and reports generated by a treatment facility will also be helpful in assessing the seasonal characteristics of the wastewater throughout the year and in determining sampling frequency.

Once a sample is taken, the sample should be maintained in the same condition as when it was collected. When it is not possible to immediately analyze collected samples, samples should be properly preserved (Table 4.2). Biological activity such as microbial respiration, chemical activity such as precipitation or pH change, and physical activity such as aeration or high temperature should be kept to a minimum. Methods of preservation include cooling, pH control, and chemical addition. Freezing is usually not recommended. The length of time that a constituent in wastewater will remain stable is related to the character of the constituent and the preservation method used. The *EPA Handbook for Sampling and Sample Preservation of Water and Wastewater* (EPA 1982) provides detailed guidelines on sampling, preservation techniques, and analysis of wastewater samples.

Table 4.2. Summary of Preservation and Holding Times.

Required Containers, Preservation Techniques and Holding Times for Water and Seawater

Name	Minimum Sample Size	Container[1]	Preservation[2]	Maximum holding time
Biological Tests				
• Coliform, total	• 500 mL	• P, G	• Cool, 4°C, 0.008% $Na_2S_2O_3$	• 6 hours
• Chlorophylls	• 100-300 mL	• P	• Cool, 4°C, 0.008% $MgCO_3$	• 8 hours before filtering
		Wrapped in aluminum foil	• Frozen –20°C (after filtration)	• Several Weeks for filters
Inorganic Tests				
• Chloride	• See method	• P, G	• None required	• 28 days
• Hydrogen ion (pH)	• 25 mL	• P, G	• None required	• Analyze immediately
• Nitrate/Nitrite	• 200 mL/100 mL	• P, G	• Cool 4°C	• 48 hours[3]
• Sulfate	• 50 mL	• P, G	• Cool 4°C	• 28 days[3]
• Phosphate	• 50 mL	• P, G	• Cool 4°C	• 28 days[3]
• Suspended Solids	• 1-4 L	• P, G	• Cool 4°C, add zinc acetate	• 7 days
• Dissolved Oxygen	• 125 mL	• BOD Bottle, Iodine determination flask	• Cool 4°C	• 7 days
• COD	• 50 mL	• P, G	• H_2SO_4 to pH < 2, cool	• 28 days
• BOD	• 50 mL	• P, G	• Cool	• 48 hours
Metals				
• Mercury	• See method	• P, G	• HNO_3 to pH<2	• 38 days in glass • 13 days in plastic
• Other Metals		• P, G	• HNO_3 to pH<2	• 6 months
Organic Tests				
• Oil and grease	• 500 mL - 1L	• G	• Cool, 4°C, H_2SO_4 to pH<2	• 28 days
• Organic carbon	• See method	• P, G	• Cool, 4°C, H_2SO_4 to pH<2	• 28 days
• PAHs	• 500 mL - 1L	• G, Teflon lined cap	• Cool, 4°C, H_2SO_4 to pH<2	• 7 days until extraction

[1]Polyethylene (P) or Glass (G)

[2]Adjust to pH<2 with H_2SO_4, HCl or solid $NaHSO_4$

[3]Can freeze and store, 48H holdin time with no preservation

Table 4.2. (Cont.)

Required Containers, Preservation Techniques, and Holding times for Snow

Name	Minimum Sample Size	Container[1]	Preservation[2]	Maximum holding time
Inorganic Tests				
• Particulates	• See method	• P, G	• Cool 4°C, add zinc acetate	• 7 days
Metals				
• Mercury	• 150 mL (melted)	• P, G	• HNO_3 to pH<2	• 38 days in glass • 13 days in plastic
• Other Metals	• 350 mL (melted)	• P, G	• HNO_3 to pH<2	• 6 months
Organic Tests				
• Oil and grease	• 500 mL-1L (melted snow)	• G, Teflon lined cap	• Cool, 4°C, H_2SO_4 to pH<2	• 28 days
• PAHs	• 500 mL-1L (melted snow)	• G, Teflon lined cap	• Cool, 4°C, H_2SO_4 to pH<2	• 7 days until extraction
• TPH	• 500 mL-1L (melted snow)	• G, Teflon lined cap	• Cool, 4°C, H_2SO_4 to pH<2	• 7 days until extraction

Required Containers, Preservation Techniques, and Holding Times for Soil/Sediment

Name	Minimum Sampling Size	Container[1]	Preservation[2]	Maximum holding time
Miscellaneous Tests				
• Grain Size	• 30 g	• P, G	• Cool 4°C	• Indefinite
• Organic carbon	• 5 g wet weight	• P, G	• Frozen –20°C	• Indefinite
Metals				
• Mercury	• 1g wet weight	• P, G	• Frozen –20°C	• 1 year
• Other Metals	• 1g wet weight	• P, G	• Frozen –20°C	• 1 year
Organic Tests				
• Oil and grease	• See method	• G, Teflon lined cap	• Frozen –20°C	• 1 year
• Organic carbon	• See method	• G, Teflon lined cap	• Frozen –20°C	• 1 year
• PAHs	• See method	• G, Teflon lined cap	• Frozen –20°C	• 1 year

[1]Polyethylene (P) or Glass (G)
[2]Adjust to pH<2 with H_2SO_4, HCl or solid $NaHSO_4$

4.2 Marine and Freshwater

Marine and freshwaters are often sampled for a range of properties in support of monitoring activities. In many cases the body of water is substantial enough and the parameters of interest are heterogeneously distributed throughout the water body so that multiple samples are required. Samples can be collected areally to cover the horizontal dimensions of the water body and/or vertically to detect gradients within the water column. These spatial collections are often needed since pollutants enter a water body at a point source and then are diluted by mixing with uncontaminated water. The areal extent of the introduced materials is often key in determining the seriousness of the contamination and what secondary effects may result. For example, introduction of sewage via an outfall in a high energy environment (extensive wave and wind action) may cause little perturbation of the surrounding marine environment because the toxicants or bacteria are rapidly diluted and/or are transported from the area by currents. In a closed bay where the water resides for long periods of time, the toxicants would tend to accumulate and increase to levels that may impact the resident biota. In the case of sewage, the introduction of organic carbon (measured by BOD and COD) could cause the resident bacteria to completely consume the oxygen in the water column producing eutrophication. The physical properties and dynamics of water bodies in general tend to result in a heterogeneous distribution of contaminants, thus there is a need for spatial sampling designs.

Water properties can be sampled using bottles to collect discrete samples or through the use of *in situ* sensors or both. Sampling equipment can be deployed by a variety of means. Key considerations in selecting sampling method are that samples are collected in a manner that ensures the safety of the sampling personnel and the integrity of the samples. This section discusses the use of water bottles to collect water samples and *in situ* instrumentation.

4.2.1 Discrete Water Sample Collection

Water-bottle samplers are relatively simple devices that generally consist of some type of cylindrical tube with stoppers at each end and a closing device that is activated by a messenger or an electrical signal. Sample bottles can also be manually filled and stoppered for snow and/or runoff sampling by directly submerging the bottle in the water to be sampled. The most commonly used samplers are the Van Dorn, Niskin, Nansen, and Go-Flo samplers. Each device samples a discrete parcel of water at a designated depth. Each sampler can be attached directly to a wire or cable and lowered to the desired sampling depth. Frequently in marine settings, multiple water samplers are fixed on a rosette frame so that several depths can be sampled during one cast or replicate samples can be taken at the same depth using electronic releases.

Prior to deployment, the stoppers of water-bottle samplers are cocked open. It is critical that the interior of the sampler and stoppers remain free from contamination. Personnel should avoid touching the insides of the sampler and/or stoppers. The interior of each water bottle sampler should be washed periodically with 10-percent hydrochloric acid (non-metallic bottles only). This should be repeated if contamination is suspected. As an alternative to the acid washing, a laboratory cleaning solution can be used. The gear should then be well rinsed with ambient water.

After cocking, the sampler is lowered to a designated depth or deployed manually by hand. The sampler is open at both ends so that water is not trapped within the device as it is being lowered into the water. Lowering the sampling bottle through any obvious surface film should be avoided if

at all possible. Once the sampler reaches the desired depth, it is allowed to equilibrate with ambient conditions for 2-3 minutes before being closed. Equilibration time is extended to five (5) minutes if thermometers are attached for the measurement of temperature.

After equilibration, the closing device can be activated by messenger or electrical signal or manually, and the sampler is retrieved. In some cases (e.g., for deep water), two samplers can be used simultaneously for each depth. A second sampler provides a backup to the primary sampler in case the latter device misfires or will not trigger or additional sample is required by the analytical method. To ensure that all subsamples at a particular depth are collected from the same water parcel, it is recommended that they all be taken as a single cast.

Once the water sampler is returned, the stoppers should be checked for complete seals. If a stopper is not properly sealed, water from the sampled depth may have been replaced by water from another depth. The water sample should be retaken if it appears the water sample has been compromised.

Acceptable water samples are subsampled as soon as possible (i.e., within 15 minutes) because appreciable delay may result in non-representative subsamples. For example, measurement of variables sensitive to biological alteration (e.g., dissolved oxygen, turbidity, color, nutrients) or settlement within the water sampler (e.g., total suspended solids, phytoplankton) can be biased by subsampling delays. Samples for dissolved oxygen measurements should be the first samples collected. If samples for other variables are not collected within 15 minutes after sample collection, the sampling bottles should be shaken prior to subsampling to ensure a homogenous water sample.

One challenge to water sampling in Antarctica is that the water bodies of interest are often covered with a substantial layer of ice. Various techniques are available for penetrating ice including chipping and/or some type of auger or drilling device. The challenge is to use these techniques in a non-contaminating and safe manner. Often the size of the water sampler will require a large access hole for through-the-ice sampling.

4.2.2 *In Situ* Sensors

A wide variety of instruments capable of measuring water column variables *in situ* are available. Most are deployed on a wire, conducting cable, or by manual placement in the water of interest. The sensors measure the variables of interest and transmit data in the form of electrical signals to external or internal recorders or meters. The simplest instruments measure conductivity (i.e., for conversion to salinity), temperature, and water pressure (i.e., for conversion to depth). Additional sensors and instrumentation can be included to measure a variety of additional variables such as dissolved oxygen, pH, transmissivity (i.e., an index of turbidity), and oxidation-reduction potential. Water transparency can be measured using an irradiance meter. Chlorophyll *a* can be measured *in situ* using a fluorometer. Generally, the operating manuals supplied with these instruments provide detailed descriptions of how to calibrate, operate, and maintain the equipment. If a particular manual lacks sufficient detail, the manufacturer should be contacted for specific guidance. An annual or semiannual intercalibration is useful for ensuring the comparability and quality of measurements.

The major advantages of *in situ* instrumentation is the ability to measure continuous depth profiles of water-column variables and the measurements are made on-site requiring no sample

return. Continuous profiling eliminates the need to select discrete sampling depths. In addition, continuous profiling can identify water-column discontinuities and plumes that may not be detected if measurements are restricted to discrete depths. *In situ* sensors also limit the need for preservation and transport of samples from the field to the laboratory. However, some sensors require substantial support facilities or equipment to maintain electronics.

4.2.3 Ice and Through-Ice Sampling

The specific program objectives will determine the sampling methods used. These objectives can range from analyzing ice cores for changes in analytes as a function of depth (time) to ice serving as an impediment to collection of water samples from below the ice.

Ice cores drilled through an ice sheet provide a sample of all the layers of snow that have accumulated over time. Ice cores can provide uniquely detailed samples on time-scales ranging from decades to several hundred thousand years. Ice cores are collected using a wide spectrum of ice drills ranging from hand augers, mechanical drills, to extensive electro-mechanical drills that are capable of retrieving cores with a 13.5 cm diameter from depths as great as 5000 meters. Ice drilling is enhanced by the use of a drilling fluid (e.g., hot water, ethanol, a mixture of aviation fuel and freon) some of which are considered contaminants. A summary of different types of ice drilling equipment typically used is provided in Table 4.3.

Following recovery of an ice core, the core must remain in a frozen state until the ice is sampled, typically in a laboratory off the ice. Once at the facility, sampling an uncontaminated portion of the core becomes important. The outside of the core has been in contact with the drilling equipment and may not be representative of the ice sheet. A number of techniques have been developed where the interior of the ice core is melted (without also melting the outer layer of the core) and the water is then collected and analyzed. These techniques are outside the scope of this manual and are available from the Greenland Ice Sheet Project (GISP), Antarctic Astronomy and Astrophysics Research Institute, International Glaciological Society (IGS), European Project for Ice Coring in Antarctica (EPICA), and the Polar Ice Coring Office of the National Institute of Polar Research (PICO).

Through-ice sampling provides a method for collecting water samples from below ice sheets. Sites should be chosen where the water is known to be deep enough to avoid stirring up bottom sediments when penetrating the ice. Depending on the thickness of the ice, a hand or motorized auger can be used. Extremely thick ice may require a more elaborate drilling system. Once the hole is open, traditional water and sediment sampling equipment can be lowered into the underlying water and samples collected. An extensive survey of a lake may require more penetrations than is desirable or feasible. In these instances, the use of divers to collect the sediment, water, and biological samples is an alternative.

4.3 Snow

Depending on the analytes of interest, various types of sample containers have been recommended for the collection of snow and ice samples. The common feature among containers for the collection of snow is that they need to be meticulously cleaned. Container lids need to have a water-proof seal for transport of the melted snow. The choice of sample containers for inorganic analytes, including trace metals and ions, are polyethylene or polypropylene (e.g., Nalgene) bottles that are first rinsed with distilled water and then soaked in distilled water for at least 24 hours. After soaking, the bottles are again rinsed with fresh

Table 4.3. Drill Types.

Drill Title	Type	Depth Capability	Core Diameter
Jiffy Auger	Internal Combustion	1 to 5 m	None
Lightweight Auger: 20 m system	Manual	20 m/50 m	7.6 cm/10.2 cm
82 mm Eclipse Drill	Electro-Mechanical	240 m	82 mm
4 inch Drill	Electro-Mechanical	353m** 500 m*	10 cm
5.2 inch Wet/Dry Drill	Electro-Mechanical	200 m - 5,000 m	13.5 cm
5.2 inch Rock Drill Attachment	Diamond Coring	50 m	1.3 inch
30 cm Dry Drill	Electro-Mechanical	200 m**	30 cm
Thermal Drill	Electro-Thermal	312 m 4,000 m**	8.7 cm
Hot Water Shot Hole Drill	Hot Water	30 m	4.0 inch hole
AMANDA Drill	Deep Hot Water	220 m 2200 m	30.0 inch hole

* Depth capability is dependent on ice temperature and strain history; usual limit is core quality or hole closure.

** Untested to specified depth.

distilled water. The samples are then dried in a clean environment and sealed until sampling. If hydrocarbons are the target analyte, glass bottles are preferred that have been washed with low residue detergent, rinsed with distilled water, rinsed with solvents (acetone to dry and methylene chloride to remove any film), and dried in a clean environment. Often the glass bottles are combusted at 450ºC if the closures will not be distorted by heat. The bottles are sealed with precombusted aluminum foil and stored until sampling. Sampling utensils also need to be meticulously cleaned by similar procedures.

The following procedures are extensive and in most instances a surficial snow sample will be adequate for detecting atmospheric deposition. However, snow and ice profiles can provide a retrospective look at contamination in the area. This method should be adapted to the needs of the individual monitoring program.

4.3.1 Sampling Procedures

Snow is often manually collected by using scrapers made of polycarbonate (Lexan) or plexiglass. Three different sizes are recommended (15 x 15 cm, 10 x 10 cm, and 10 x 2 cm) to make the sampling more efficient. Beveled edges on several sides facilitate the collection of snow by scraping. A non-beveled handle makes the scrapers easier to handle. Scrapers are cleaned using methanol and a small brush for scrubbing. The scraper should be rinsed with purified water and allowed to soak for 48 hours in purified water. After soaking the scrapers are rinsed three times and placed in a new bath of purified water for 24 more hours of soaking. The

scrapers are then rinsed and dried in a clean environment and stored in sealed containers bagged in polyethylene bags. It is recommended that the field technician wear polyethylene gloves during the sampling. Scrapers should be carefully rinsed and cleaned between samplings. In cases were very low levels are expected, clean suits (polyester or tyvek) and particle masks may be required to prevent contamination of the snow samples.

In addition, it is useful to also have a plastic shovel to initially clean the sampling wall, a metal shovel for snowpit excavation, a tape measure to accurately record the depth of the sampling, and a snow density kit to measure snow accumulation rates.

4.3.2 Snowpits

In locations that are primarily snow covered, it is recommended that sampling be done by excavating snowpits to ensure undisturbed samples are retrieved.

After a snowpit site has been selected, all sampling equipment should be at the site to allow for immediate sampling of the snowpit upon completion of the excavation. First, the direction of the wind is determined and a clean, dedicated metal shovel is used to define an edge perpendicular to the wind direction about 1.5 to 2 meters long. This will be the sampling wall. Nothing should be placed upwind of the site. Also during digging, nothing (e.g., hands, body, etc.) must touch the wall to be sampled, other than the shovel. The upper 10-50 cm will probably be most easily removed using the shovel. The top of the snowpit should measure ~2 x 1.5 meters to give sufficient room for working in the pit. Once loose snow is removed from the upper 10-50 cm, a snow saw can be used to cut blocks and using the pointed or square shovel break the blocks free. All spoils (excavated snow) from the pit must be placed downwind from the sampling wall. If there is blowing snow, then a snow wall may be built on the upwind side of the pit. This should be done using blocks cut out of the pit. If a wall is built, it should remain 1 meter from the sampling wall. Once the wall is built no one should be upwind of the pit. Final depth of the snowpit should be ~2.1 meters (for a 2 meter snowpit). To help facilitate the sampling of the pit a large, firm block can be left in the bottom of the pit to stand on while sampling the top of the pit.

4.3.3 Snowpit Sampling

Two people are recommended for sampling the snowpit: one person who samples the pit and the other person assists with bottles and note taking. Upon snowpit excavation and completion, it is recommended that the sampler wears a clean suit, particle mask, and polygloves. The other person should be dressed in clean non-particulating clothing as well as a particle mask and clean dedicated gloves. Using the clean shovel, a ~50 cm wide section of the sampling wall is removed and at least 15 cm into the wall from top to bottom of the sampling wall. Once this is done, the spoils should be removed from the pit and the plastic shovel should be placed in a clean plastic bag for use in the next snowpit.

The sampler should replace the used polygloves. Next, one of the 15 x 15 cm scrapers is used to remove ~5 more centimeters from the cleaned shovel surface. This scraper can then be inserted into the corner of the cleaned sampling wall and act as a shelf to place the sample cup lid while sampling. Next, one of the 10 x 10 cm scrapers is used to clean the sampling surface back several centimeters down (about 50 cm from the top of the pit). This step is repeated as

samples are collected down the wall. The 10 x 10 cm scraper can be inserted into a cleaned section of the wall to keep clean for the next 50 cm of sampling. A measuring tape can be hung down the wall to accurately determine depth of sampling. A scraper at the surface will help alleviate the tape cutting into the surface.

The correct sampling template corresponding to the desired sample interval (ideal sample interval is 8-10 samples/year) is selected. By keeping the index marker parallel to the surface, the wall is marked using the template. Several passes may be necessary to get good markings on the snow wall. The template is placed into a cleaned section of the wall. The wall is now ready for sampling. The helper should then hand the sampler a sample cup with the lid pre-loosened. The sample cup lid is removed and placed on the "shelf" made by the 15 x 15 scraper with the inside of the lid down. One of the 20 x 2 cm scrapers is used to remove ~1 cm just above the line made by the template. Then the sample cup is placed lightly against the wall and filled with snow using the scraper. The scraper is placed into a clean section of the wall and the lid is put back on the cup. Cups are exchanged with the helper and collection is contined until one is about 3-4 samples from the marks from the template. Using the previous marks as a guide, additional marks are made down the cleaned snow wall. The 10 x 10 scraper is used to pre-clean the wall once the ~50 cm cleaning is reached. Using the measuring tape, true depth can be cross-correlated with sample depth. As the sampling depth in the snowpit is increased, the snow hardness will increase. It may be necessary to use the 20 x 2 cm scraper like a chisel in order to break up the snow.

Sample cups must be checked each time to assure they are in order. The helper should pre-loosen the lids to the sample cups before handing it to the sampler. Once the sample is collected and cup returned to helper, it is necessary to tighten the lid as much as possible. Samples must be placed out of the direct sun to prevent melting. The helper records notes about the snowpit which include description of the snow from the sampler, container numbers, general weather and sampling conditions and miscellaneous notes related to sampling (e.g., stratigraphy, grain size, etc.).

Other guidance includes:

- while sampling, check polygloves often for rips or tears and replace as necessary;
- remember, 2 pairs of polygloves work better than one pair;
- record good notes on snowpit excavation and measurement;
- always have an additional "clean" section on the pit wall (this section can be used to clean tools that may drop to the pit floor, the snow is a clean, abrasive cleaner); and
- remember to keep hands away from the particle mask (if it touches the polygloved hands, the polyglove should be replaced; the mask concentrates breath which has very high concentrations of ions measured in the snowpack).

4.3.4 Stratigraphy and Physical Studies

Density and physical stratigraphy can be recorded after the sampling of the snowpit. Parameters such as layer thickness, hardness, color, crystal size and shape, location and thickness of ice or debris bands should be recorded. Photographs of the pit wall should be taken to record the visible structure and layering.

4.3.5 Snow Pack Depth

Snow pack depth gives the sampler an idea of the snow's physical properties. Because many factors can play a role in snow redistribution, such as: annual temperature, wind, energy or moisture transfer, and physiography, a snow depth measure gives the sampler an idea of how these factors effect snow pack depth and distribution.

To measure snow pack depth:

- Take 2 sections of aluminum poles approximately 1/4 inch in diameter. The first section should be 6 meters in length and the second section should be 3 meters in length. The sampler or "snow probe" should have the aluminum poles processed so that the 3 m subsection can be fitted onto the 6 m section. These poles should have hatch markings every centimeter so that the sampler can measure snow depth.
- While on the snow pack, place the pole into the snow pack in a straight perpendicular fashion until a hard surface hampers pole movement into the pack.
- Finally, note the final measurement, or the cm hatchmark at the snow line.
- Repeat this process at nine (9) other randomized points in the snow pack.

4.4 Sediments and Soils

The most common sampling device for marine surficial sediments is some type of bottom grab sampler. However, various coring devices (e.g., box corer) are also used for soils and sediments. The primary criterion for a sediment sampler is that it consistently collects undisturbed samples to the required depth without contaminating the samples. An additional criterion is that the sampler can be easily handled in the field. An otherwise acceptable sampler may yield inadequate sediment samples if it is too large, heavy, or awkward to be properly handled.

Collection of undisturbed sediment/soil requires that the sampler:

- creates a minimal disturbance of adjacent soil/sediment;
- closes to form a leakproof seal after the sample is taken;
- prevents losses and excessive sample disturbance during retrieval and processing; and
- allows easy access to the sample surface.

Most grab samplers have open upper faces that are fitted with flaps. For manual sampling, various spatulas or other utensils are employed. These utensils may be coated by various materials depending on the analytes of interest. For marine sediments collected by grab samplers, the flaps are open to minimize the bow wake during descent, whereas upon ascent the flaps are closed to prevent sample washout. Some box-corers have solid flaps that are clipped open upon descent and snap shut after the corer is triggered. Although most samplers seal adequately when new, the wear and tear of repeated field use eventually reduces their sealing ability (e.g., through chipped or improperly aligned jaws). A sampler should therefore be properly maintained and monitored constantly for proper operation and to ensure minimal sample leakage. If unacceptable leakage occurs or the sampler malfunctions in any manner, the sampler should be repaired or replaced. Also, it is prudent to have a backup sampler if the primary sampler malfunctions.

Penetration below the sediment surface is usually desired. Generally, it is better to penetrate below the desired sample depth to minimize sample disturbance when the sampling device closes. Penetration depth of most sampling devices varies with sediment texture; it is greatest in fine-grained sediments and least in coarse-grained sediments. Sampling devices generally rely upon either force, gravity, or a piston mechanism to penetrate. In most cases, penetration depth can be modified by adding or removing steel or lead weights from the samplers. Thus, it is optimal to use a sampler that has a means of weight adjustment. If a sampler cannot consistently achieve the desired penetration depth, an alternate device should be used.

The sampler should be retrieved in a manner that minimizes sample disturbance. Once the sampler is secured, it is essential that the surface of the sample be made accessible without disturbing the sample. Most grab samplers have hinged flaps on their upper face for this purpose. The openings in the upper face of the sampler should be large enough to allow convenient sub-sampling of the sediment surface. If an opening is too small, the sample may be unduly disturbed as the field member struggles to take a subsample. Other sampling devices may directly sample the soil/sediment surface or be extruded by a plunger-like device.

After the sampler is retrieved, the sediment sample should be inspected carefully before being accepted. The following acceptance criteria should be satisfied:

- the sampler is not overfilled with sample such that the surface is pressed against the top of the sampler;
- the overlying water is present (indicates minimal leakage);
- the overlying water is not excessively turbid (indicates minimal sample disturbance);
- the sediment surface appears to be relatively undisturbed (i.e., lack of channeling or sample washout); and
- the desired penetration depth was achieved (i.e., 4-5 cm for a 2-cm-deep surficial sample).

If a sample does not meet all of these criteria, it should be rejected and discarded away from the sampling station. The required depth of penetration needs to be established based on the study goals.

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

Once the overlying water has been removed, the surficial sediment is subsampled. It is recommended that subsamples be taken using a flat scoop. This device will allow a relatively large subsample to be taken accurately to a depth of 2 cm. A curved scoop is not recommended because it does not sample a uniform depth.

Finally, sample contamination during collection must be avoided. All sampling equipment (e.g., scoops, containers) should be made of non-contaminating material, and should be cleaned appropriately before use. It is recommended that all objects coming in contact with the sample be made of glass, stainless steel, or PTFE (i.e., polytetrafluoroethylene; e.g., Teflon or equivalent). To avoid contamination, all sampling equipment should be cleaned in sequence with site seawater, pesticide-grade acetone, and pesticide-grade methylene chloride prior to initial use and between use for each station. The methylene chloride should be allowed to evaporate prior to using the equipment. Recommended sampling procedures, materials, and preservation conditions are summarized in Table 4.2.

CHAPTER 5
STATION ACTIVITIES

Any monitoring program needs to compare the observations collected to quantifiable measures of human activities in the study area to develop a plan for management. The data collected as part of monitoring must be interpreted within the context of a general description and understanding of the stations environs and the landscape features surrounding it. It is also important to gather the "vital statistics" related to human activities in the area. This can be accomplished through gathering of information on the number, timing duration, and intensity of various activities that occur within the area of concern. These quantifiable measures of human presence are then used to qualitatively understand changes in the physical setting around stations. These are the activities to be managed in a way to minimize or eliminate perceived or real deterioration in the resources and values that are to be protected. While many of the techniques described so far are related to the release of chemicals or biological agents into the environment, physical disturbance, landform alterations, and changes in snow cover and drainage patterns can be equally important in understanding and predicting impacts that result from the presence of humans. The physical setting is best quantified through thematic mapping of key system attributes. These provide a framework within which the observed change can be understood, and cause and effect inferred by relating the changes to activities taking place within the study area.

5.1 Station Performance

In the deliberations during the SCAR/COMNAP Workshops (Kennicutt et al. 1996), a fundamental recognized feature of Antarctica was that the import and export of materials was controlled by those parties operating on the continent. This more or less "closed" system allows for a first order assessment: 1) of the quantities of materials being introduced to the area, 2) the maximum amounts released or left behind based on a balance of inputs and outputs, and 3) the activities that take place and where they take place. This is called a desk top assessment and provides a general framework within which monitoring observations can be interpreted. This inventory approach was seen as a first critical step to indicate which activities are of potential concern, providing guidance on the content and design of a monitoring program.

Station performance can be quantified based on station population, quantity of fuel imported and used, energy production and utilization, type and quantity of motorized vehicles, solid and wastewater disposal, and spills. It is recommended that each operator provide a routine inventory of the following activities:

- number of people, their location and the duration of their stay;
- number of tourists, their location, and the duration of their visit;
- fuel usage by fuel type, mechanical equipment, and its location;
- energy generation and usage;
- amount, location, type and duration of vehicle traffic;
- amount, location, type and duration of aircraft traffic;
- details of any construction projects;
- timing, amount, composition and location of solid non-hazardous waste disposal;
- timing, amount, composition, and location of sewage and grey water disposal;

- timing, amount, composition and location of hazardous waste disposal;
- timing, amount, composition and location of fuel and chemical spills; and
- timing, extent, and types of fires.

Often retrospective gathering of these types of information provides a longer-term view of activities at the site and its development over time. Also at some of the larger stations subject to national laws, information may be collected to address compliance issues. These statistics should be routinely gathered and used to develop a more complete picture of the location being monitored. While it may be difficult to determine the exact geographic location and intensity of various activities, such information provides an overall framework or "sphere of influence" to frame any observed changes in the station operations and the surrounding environs over time. In order to be directly comparable to the classes of discharges referred to in the Protocol, the general disposal categories that need to be quantified are sewage and domestic liquid wastes, other liquids and chemicals (including fuels and lubricants), solids to be combusted (discontinued at most sites), other solid wastes, and radioactive materials. Each national program should develop a complete understanding of what is being imported to the site, what is consumed on site, and what is discharged or released at the site on at least an annual basis. This information will provide a first order estimate of the boundaries of expected human influences on the environment. Other impacts such as physical disturbance are at least semiquantitatively related to the types, the duration and the intensity of activities conducted in support of science at Antarctic stations. Many lists of activities and potential impacts have been compiled by various workshops and organizations and provide guidance for national managers to assess the specifics of their operations.

5.2 Aerial Photography

Monitoring of the terrestrial environment can be aided by interpretation of aerial photographs of the study area if air support is available. At locations that cannot be surveyed by aerial photography, "on-the-ground" surveys to produce thematic maps of the area can be a substitute and in some instances remote sensing techniques may be applicable. Aerial photography can be used to map snow cover, physical disturbance, and land usage. These interpretations are then "groundtruthed" with on-the-ground point sampling to verify the identification of features in the photographs. Visual recognition of the aerial extent of disturbance as defined by the alteration of landscape can be quantified as vegetation coverage, snow cover, and topsoil loss or erosion.

In an ideal situation, low altitude aerial photographs are taken at a scale of 1:2500 depending on the size of the site being monitored. This spatial resolution, or grain, provides images that can be developed into high quality maps of the area. This type of photography provides resolution (□1 meter) which is sufficient for establishing the expansion or contraction of the snow-field or detection of increased or decreased physical disturbance. Photography provides detailed base maps for posting other types of information gathered during monitoring and assists in study design and station location for point data collection. The technical issue of greatest concern in photographic data is to provide a precise geographic context to allow for accurate reoccupation of the same location year after year. This is referred to as georectification and is aided by precise positioning systems such as Global Positioning System (GPS).

Information is also most effectively utilized within the context of a geographic information system (GIS) that allows for overlaying of many different types of data including contours of variable distributions and photographs. A second issue important in developing thematic maps is the development of an accurate and repeatable classification of the images being analyzed. The topography of the area is important in understanding possible pollutant transport mechanisms and pathways and to assess changes in land forms over time. The topographic information is most effectively quantified by the development of a digital elevation model (DEM) which is a numerical representation of the slope and elevation at many points across the study area. The issue of proper classification of images is directly addressed by on-the-ground surveys of relevant system attributes.

The variables of interest for thematic maps include soil coverage, rock outcrops, vegetation coverage (possibly by species), physical disturbance (type and depth), the extent of snow and ice fields, land usage, and the presence and location of important biological resources. Land usage is quantified as the area occupied by buildings, storage tanks, roads, landfills, snow disposal areas, pipelines, and excavations. Changes in development at the location can be discerned over long time periods from historical photographs if available, and of appropriate quality. Again, this provides a longer time-frame view of the development of the station and possibly provides an explanation for changes observed in the surrounding landscape.

For groundtruthing of aerial photography and remote sensing images, data should be collected along grids within the study area. Remote sensing may be useful in determining the dynamics of snow and ice fields in the area. In order to confirm interpretations of the photographs, vegetation coverage, snow and ice coverage, physical disruption, soil compaction, permafrost depth, and possible water infiltration rates can be measured at specific locations throughout the study area. In addition, the indicators of impact previously described such as contaminants and other basic soil parameters such as grain size and carbon content are quantified by discrete sample collection and analysis. Once confidence in the interpretation of the photography is established, aerial photography can provide a useful reconnaissance tool when putting people in the fields may be too resource intensive. Aerial photography can also be electronically stored, thus providing documentation of the evolution of the site being studied from a broad, region-wide perspective that discrete sampling may not be able to provide.

SECTION B

ANALYTICAL TECHINQUES

CHAPTER 6.
WASTEWATER/SEAWATER/FRESHWATER

The analysis of waters of various types is an important element of a monitoring programIn addition to air, water is the main agent of contaminant transport in both marine and terrestrial settings. Water can transport contaminantsas run-off from the land surface to adjacent aquatic environments. In many instances, dischargesare in liquid form and are often introduced directly into receiving waters, e.g. sewage outfalls. The following methods are appropriate for wastewaters, seawater and freshwater. In some instances, the higher concentrations of analytes expected in wastewaters may require modification of the methods proposed (often dilutions of samples or the use of higher concentrations for detector calibration). Waters are typically collected using a range of samplers and require various types of preservation to maintain sample integrity until the analysis is conducted (see Chapter 3). The monitoring parameters of interest include total suspended solids (TSS), dissolved oxygen (DO), nitrate (NO_3), phosphate (PO_4), acidity, pH, conductivity, temperature (T), coliform bacteria, and phytoplankton biomass. In addition, two measurements of the capacity of waters and particulates to deplete oxygen from aqueous samples are included: biological (BOD) and chemical oxidation demand (COD). The recommended methods have been widely used for many years, indicating the importance of water quality in establishing the impact of activities related to the presence of humans.

Some of the parameters discussed in this chapter can only be measured on site due to the limited time during which the constituents of interest remain in their original forms. Certain constituents can rapidly degrade, diminish or transform after removal from their *in situ* location. Each method is described with regard to sampling, preservation and storage requirements, and limitations. In those cases where on-site facilities are limited, other possible approaches are discussed if available. In other cases, despite the appropriateness of the measurement to monitor activities at the station, it may not be possible to include the parameter in a monitoring program.

6.1 Total Suspended Solids (TSS)

Total suspended solids (TSS) is a measure of any floating or suspended solids. TSS is an important source of water pollution. Suspended solids consist of inorganic (e.g., silts, runoffs, clays, etc.) and organic materials (e.g., algae, zooplankton, bacteria, and detritus). TSS is present in water and is present in discharged materials (i.e., sewage). Particulates contribute to the turbidity, or cloudiness, of water. Waters with high particulate loads are obvious because of their "muddy" appearance. Particulate matter can also be produced *in situ* by biological organisms and from flocculation of dissolved constituents. Reduced transparency in waters can inhibit the growth of biological organisms that are dependent on sunlight for energy (i.e., phytoplankton). Some portion of suspended solids are ultimately deposited in sediments and can cause changes in sediment distributions, alter biological patterns, and transport contaminants.

Particulate matter is considered to be stable in its original medium (water) for seven days if cooled to 4ºC. Samples that are stored beyond seven days can be subject to artifacts due to dissolution of particulate matter or flocculation of dissolved material. It is best if waters are filtered on-site within seven days of collection. In most cases where the particulate matter amount is low, gravity is sufficient for filtration. Once on the filter, particulates can be stored for long periods of time, however it is best that water is removed from the filter by drying. Drying must be done in a clean setting so that foreign materials are not deposited on the filter. There are

continuous reading sensors that give an indication of the concentration of particulates but they are generally used in large bodies of water and require some type of energy supply to operate.

The need to determine TSS as part of an Antarctic monitoring programs will be dependent on several issues. The first obvious consideration is whether there is water, and especially moving water, in close proximity or within the area to be monitored. Water can be in the form of lakes, streams, and/or adjacent marine water along the continental coast. If the station or camp is far removed from significant accumulations of water most of the water quality parameters discussed would not be of interest. TSS in streams would be important if activities within the catchment area alter the particulate load of the streams disrupting natural sedimentation patterns in the associated water accumulations. Increases in TSS can be caused by changes in drainage patterns, physical disturbance of land forms and increases in erosional removal of materials at the site and do not necessarily involve the introduction of foreign particulate matter. TSS also can be introduced by discharges into lake or coastal waters. In general, discharge of sewage or other effluents is not allowed into lakes. Therefore the most likely case of interest, as far as monitoring is concerned, is the discharge of effluents to adjacent coastal waters including sewage, grey waters, and waters from desalination plants. The importance of these discharges will be dependent on the size of the station's population, the duration of the station occupation, and the methods employed to treat sewage and other fluids before discharge. All of these factors need to be considered when deciding whether TSS is an important parameter to measure. As was mentioned, particulates can have a direct effect through organic enrichment followed by eutrophication or lowering of oxygen levels but can also impact local sediment characteristics and in extreme cases bury local benthic communities. Also, depending on the origin of the TSS, these particles can have enhanced contaminants levels. For example, runoff that traverses the station area and picks up trace metal and hydrocarbon pollutants is then transported into adjacent water bodies.

The following method is adapted from: 18th Edition Standard Methods for the Examination of Water and Wastewater; Total Suspended Solids Dried at 103-105°C (TSS); Greenberg, A.E., Clesceri, L.S., and A.D. Eaton (Eds.), APHA: Washington, 1992.

"Apparatus, Materials, and Reagents - Suspended Solids"

- **Muffle Furnace** for operation at 500 ± 50ºC
- **Dessiccator**, provided with a desiccant containing a color indicator of moisture concentration or an instrumental indicator.
- **Drying Oven** - for operation at 103 to 105ºC
- **Analytical Balance** - capable of weighing to 0.1 mg.
- **Magnetic Stirrer** with TFE stirring bar.
- **Wide-Bore Pipets**.
- **Glass-fiber filter disks** without organic binder.
- **Filtration apparatus** - One of the following, suitable for the filter disk selected:
  1. Membrane filter funnel.
  2. Gooch crucible, 25-mL to 40-mL capacity, with Gooch crucible adapter.
  3. Filtration apparatus with reservoir and coarse (40- to 50-μm) fritted disk as filter support.
- **Suction Flask** of sufficient capacity for sample size selected.

6.1.1 Sample Preparation

A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried at 103 to 105ºC to a constant weight. The increase in the weight of the filter is referred to as the total suspended solids (TSS). If the suspended material clogs the filter and prolongs filtration, the analysis should be repeated using smaller volumes.

It is important to:

- exclude large floating particles or submerged agglomerates of nonhomogeneous materials from the sample if their inclusion is not desired;
- limit the sample size to yield no more than 200 mg of residue because excessive residue on the filter may form a water-entrapping crust; and
- thoroughly wash the filter to ensure removal of dissolved material especially for samples high in dissolved solids.

Prolonged filtration times resulting from filter clogging may produce erroneously high results owing to the capture of increased colloidal materials on the clogged filter.

To prepare a glass-fiber filter disk:

- insert the disk with the wrinkled side up in a filtration apparatus;
- apply the vacuum and wash the disk with three (3) successive 20-mL portions of reagent-grade water;
- continue suction to remove all traces of water and discard the washings;
- remove the filter from the filtration apparatus and transfer it to an inert planchet or small metal disk taking care to prevent the dried filter from adhering to the planchet; and
- place in drying oven then desiccator and weigh.

Alternatively, weigh the dried filter and planchet together before and after filtration. Filter material that sticks to the dish should be included with the filter weight.

6.1.2 Sample Processing

Choose sample volumes that yield between 10 and 200 mg of dried residue. If more than ten (10) minutes are required to complete the filtration, increase the filter size or decrease the sample volume. When very low amounts of total suspended solids are present (less than 10 mg/L), less residue may be collected. For low TSS samples use a high-sensitivity balance (0.002 mg). For non-homogeneous samples, such as raw wastewater, use a large filter to permit the filtering of a representative sample.

To analyze a sample:

- assemble the filtering apparatus and filter and begin suction;
- wet the filter with a small volume of reagent-grade water to seat it;
- while stirring the sample with a magnetic stirrer, pipet a measured volume onto the seated glass-fiber filter;

- wash with three successive 10-mL volumes of reagent-grade water allowing for the complete drainage between washings and continue the suction for about three (3) minutes after filtration is complete (samples with high dissolved solids may require additional washings);
- carefully remove the filter from the filtration apparatus and transfer it to an aluminum or stainless steel planchet as a support;
- dry for at least one (1) hour at 103 to 105^{o}C in an oven;
- cool in a desiccator to balance temperature, and weigh; and
- repeat the cycle of drying, cooling. desiccating, and weighing until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg, whichever is less.

6.1.3 Calculations

The following calculation provides the concentration of total suspended solids in mg/L:

mg total suspended solids/L = [(A-B) x 1000]/sample volume in mL

where:

A = weight of the filter + the dried residue in mg and
B = weight of the filter in mg

Duplicate determinations should agree within 5% of the average.

PERFORMANCE

As a guide, the following estimates of precision have been observed for TSS measurements: the standard deviation was 5.2 mg/L (coefficient of variation 33%) at 15 mg/L, 24 mg/L (10%) at 242 mg/L, and 13 mg/L (0.76%) at 1707 mg/L in studies by two analysts of four sets of 10 determinations each. Single-laboratory duplicate analyses of 50 samples of water and wastewater were made with a standard deviation of differences of 2.8 mg/L.

STANDARD REFERENCE MATERIALS

None Known.

6.2 Dissolved Oxygen (DO)

Dissolved oxygen (DO) is the amount of oxygen in solution in either wastewater, seawater, or freshwater. Dissolved oxygen is an important monitoring parameter determined in aquatic systems since oxygen is a requirement for metabolism by aerobic organisms. Oxygen influences aqueous inorganic chemical reactions as well. Oxygen enters natural waters as a product of photosynthesis of aquatic biota and by the dissolution of atmospheric oxygen across the air-water interface. The concentration of oxygen in water is dependent on the water temperature (colder water holds more oxygen), salinity (freshwater holds more oxygen than does salt water), and atmospheric pressure.

Conditions present in Antarctic lakes with permanent ice cover, such as those found in the Dry Valleys, make conventional oxygen measurements difficult. The near surface waters are

frequently highly supersaturated with oxygen, exceeding the capabilities of commercially available oxygen sensors. The bottom waters of these lakes can be highly saline, exceeding the capabilities of commercially available oxygen sensors and introducing artifacts into the standard Winkler titration. These problems have been addressed by Downes et al. (1995).

Oxygen samples need to be carefully collected, stored and analyzed due to oxygen's post-collection susceptibility to biological activity, degassing to the atmosphere, and contamination with non-indigenous oxygen. After being "fixed" the sample can be stored for seven (7) days. Alternatively, kit type methods are available that give less precise data but can be used to indicate general oxygen conditions. In addition, various probes can be used to measure oxygen. Each probe or sensor has its own operational parameters and present differing degrees of difficulty in obtaining measurements in the field. If it is decided that on-site measurement of oxygen by a kit probe or sensor is the preferred method then each manufacturers instrument must be evaluated within the context of the conditions under which the measurements will be taken.

As for TSS, oxygen levels in water will only be important for Antarctic monitoring programs when water bodies are in close proximity to the area being monitored or it is known that down-stream water flowing through the station empties into a body of water. The most likely scenario where oxygen levels would be expected to be influenced by human activities is during the discharge of effluents. As will be explained in the following sections, discharged fluids can contain both biological and chemical agents that consume oxygen. As for other effluent related indicators, the size of the station population, the type and method of effluent discharge, and the pretreatment methods will be important in determining whether there is a potential for depletion of oxygen in any water bodies within the area of interest.

6.2.1 Modified Winkler Method

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- **Sample bottles-300 mL ± 3 mL capacity** BOD incubation bottles with tapered ground glass pointed stoppers and flared mouths.
- **Pipets**-with elongated tips capable of delivering 2.0 mL ± 0.10 mL of reagent.
- **Manganous sulfate solution**. Dissolve 480 g manganous sulfate ($MnSO_4 \cdot 4H_2O$ in distilled water and dilute to 1 liter. Alternatively, use 400 g of $MnSO_4 \cdot 2H_2O$ or 364 g of $MnSO_4 \cdot H_2O$ per liter. When uncertainty exists regarding the water of hydration, a solution of equivalent strength may be obtained by adjusting the specific gravity of the solution to 1.270 at 20°C.
- **Alkaline iodide-azide solution**. Dissolve 500 g of sodium hydroxide (NaOH) or 700 g of potassium hydroxide (KOH) and 135 g of sodium iodide (NaI) or 150 g of potassium iodide (KI) in distilled water and dilute to 1 liter. To this solution add 10 g of sodium azide (NaN_3) dissolved in 40 mL of distilled water.
- **Sulfuric acid**. Concentrated.
- **Starch solution**. Prepare an emulsion of 10 g soluble starch in a mortar or beaker with a small quantity of distilled water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight.

Use the clear supernatant. This solution may be preserved by the addition of 5 mL per liter of chloroform and storage in a 10°C refrigerator. Dry, powdered starch indicators such as "thyodene" may be used in place of starch solution.

- **Potassium fluoride solution**. Dissolve 40 g $KF \cdot 2H_2O$ in distilled water and dilute to 100 mL.
- **Sodium thiosulfate, stock solution**, 0.75 N: Dissolve 186.15 g $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 mL chloroform.
- **Sodium thiosulfate standard titrant**, 0.0375 N: Prepare by diluting 50.0 mL of stock solution to 1 liter. Preserve by adding 5 mL of chloroform. Standard sodium thiosulfate: exactly 0.0375 N is equivalent to 0.300 mg of DO per 1.00 mL. Standardize with 0.0375 N potassium biiodate.
- **Potassium biiodate standard**, 0.0375 N: For stock solution, dissolve 4.873 g of potassium biiodate ($KH(IO_3)_2$, previously dried 2 hours at 103°C, in 1000 mL of distilled water. To prepare working standard, dilute 250 mL to 1000 mL for 0.0375 N biiodate solution.
- **Standardized sodium thiosulfate**: Dissolve approximately 2 g (± 1.0 g) KI in 100 to 150 mL distilled water; add 10 mL of 10% H_2SO_4 followed by 20.0 mL standard potassium biiodate. Place in dark for 5 minutes, dilute to 300 mL, and titrate with the standard sodium thiosulfate to a pale straw color. Add 1-2 mL starch solution and continue the titration drop by drop until the blue color disappears. Run in duplicate. Duplicate determinations should agree within ± 0.05 mL.
- **Phenylarsine oxide** (PAO) may be used as an alternative to the sodium thiosulfate. This is available, already standardized, from commercial sources.

The Modified Winkler Method is applicable for use with most waters that contain nitrate-nitrogen and not more than 1 mg/L of ferrous iron. Other reducing or oxidizing materials should be absent. If 1 mL of fluoride solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100 to 200 mg/L ferric iron. The Dissolved Oxygen (DO) Probe technique below gives comparable results.

The Modified Winkler Method is not applicable under the following conditions: (a) samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite; (b) samples high in suspended solids; (c) samples containing organic substances which are readily oxidized in a highly alkaline solution, or which are oxidized by free iodine in an acid solution; (d) untreated domestic sewage; (e) biological flocs; and (f) where sample color interferes with endpoint detection. In instances where this method is not applicable, the DO probe should be used.

The sample is treated with manganous sulfate, potassium hydroxide, and potassium iodide (the latter two reagents combined in one solution) and finally sulfuric acid. The initial precipitate of manganous hydroxide, $Mn(OH)_2$, combines with the dissolved oxygen in the sample to form a brown precipitate, manganic hydroxide $MnO(OH)_2$. Upon acidification, the manganic hydroxide forms manganic sulfate which acts as an oxidizing agent to release free iodine from the potassium iodide. The iodine, which is equivalent to the dissolved oxygen in the sample, is then titrated with sodium thiosulfate or phenylarsine oxide (PAO).

The following method is adapted from: US EPA. 1998. Understanding Environmental Methods: Analytical Methods, Environmental Analysis, and Data Evaluation EPA Method 360.2 (Approved for NPDES, Issued 1971); TITLE: Oxygen, Dissolved (Modified Winkler, Full-Bottle Technique).© Genium Publishing Corporation 1996, Schenectady, NY 12304.

6.2.1.1 Sample Preparation

Collect the sample in a 300 mL BOD incubation bottle or an equivalent container. Special precautions are required to avoid entrainment or solution of atmospheric oxygen or loss of dissolved oxygen. Care must be taken to prevent turbulence and the formation of bubbles when filling the bottle. Oxygen samples should be transferred to the bottle using seasoned plastic tubing (e.g., Tygon or equivalent) filling the bottle from the bottom. At the time of sampling, the sample temperature is precisely recorded. Do not delay the determination of dissolved oxygen in samples having an appreciable iodine demand or containing ferrous iron. If samples must be preserved either method below, may be used:

- Add 2 mL of manganous sulfate solution and then 2 mL of alkaline iodide-azide solution to the sample. Both reagents must be added well below the surface of the liquid. Stopper the bottle immediately and thoroughly mix the contents. The sample should be stored at the temperature of the collection water, or seal the stopper with water and keep it at a temperature of 10 to 20°C, in the dark. Complete the procedure by adding 2 mL H_2SO_4 at the time of analysis.

- Add 0.7 mL of conc. H_2SO_4 and 1 mL sodium azide solution (2g NaN_3 in 100 mL distilled water) to the sample. Store sample as above. Complete the procedure using 2 mL of manganous sulfate solution, 3 mL alkaline iodide-azide solution, and 2 mL of concentrated H_2SO_4 at the time of analysis. If either preservation technique is employed, complete the analysis within hours of sampling.

6.2.1.2 Sample Processing

To process the sample for DO:

- Add 2 mL of the manganous sulfate solution to the sample bottle followed by 2 mL of the alkaline iodide-azide solution, well below the surface of the liquid; stopper with care to exclude air bubbles; and mix well by inverting the bottle several times.
- When the precipitate settles, leaving a clear supernatant above the manganese hydroxide floc, shake again.
- When the settling has produced at least 200 mL of clear supernatant, carefully remove the stopper and immediately add 2 mL of conc. H_2SO_4 allowing the acid to run down the neck of the bottle, re-stopper, and mix by gentle inversion until the iodine is uniformly distributed throughout the bottle.

- Transfer the entire bottle contents by inversion into a 500 mL wide mouth flask and titrate with 0.0375 N thiosulfate solution (0.0375 N phenyarsine oxide (PAO) may be substituted as titrant) to pale straw color.
- Add 1-2 mL of starch solution or 0.1 g of powdered indicator and continue to titrate to the first disappearance of the blue color.
- If ferric iron is present (100 to 200 mg/L), add 1.0 mL of KF solution before acidification. Occasionally, a dark brown or black precipitate persists in the bottle after acidification. This precipitate will dissolve if the solution is kept for a few minutes longer than usual or, if particularly persistent, a few more drops of H_2SO_4 will cause dissolution.
- Complete the analysis within 45 minutes.

6.2.1.3 Calculations

Each mL of 0.0375N sodium thiosulfate (or PAO) titrant is equivalent to 1 mg DO when the entire 300 mL bottle contents are titrated. If the results are desired in milliliters of oxygen gas per liter at 0°C and 760 mm pressure multiply mL/L DO by 0.678.

$$\text{mL/L DO} = [(P - p) \times 0.678] / (35 + T)$$

Equations for correcting the solubilities to barometric pressures other than mean sea level can be found. The solubility of DO in distilled water at any barometric pressure, P (mm Hg), temperature, ºC, and saturated vapor pressure, p (mm Hg), for the given T, may be calculated between the temperatures of 0 and 30°C by:

Between 30ºC and 50°C by:

$$\text{mL/L DO} = [(P - u) \times 0.827] / (49 + T)$$

Actual Normality of standard sodium thiosulfate:

$$\text{N sodium thiosulfate} = \frac{0.0375 \text{ x } 20.0 \text{ mL potassium biiodate (0.0375 N)}}{\text{Volume (mL) sodium thiosulfate}}$$

Dissolved Oxygen Conentration (mg/DO/L)

$$\text{mg DO/L} = \frac{\text{N sodium thiosulfate x Volume (mL) sodium thiosulfate x 8000}}{\text{Volume of container (mL)}}$$

PERFORMANCE

Exact data are unavailable on the precision and accuracy of this technique; however, reproducibility is approximately 0.2 mg/L of DO at the 7.5 mg/L level due to equipment tolerances and uncompensated displacement errors.

6.2.2 Membrane Electrode Method

The probe method for dissolved oxygen is recommended for those samples containing materials which interfere with the modified Winkler procedure such as sulfite, thiosulfate,

polythionate, mercaptans, free chlorine or hypochlorite, organic substances readily hydrolyzed in alkaline solutions, free iodine, intense color or turbidity and biological flocs. The probe method is recommended as a substitute for the modified Winkler procedure in monitoring of streams, lakes, outfalls, etc., where it is desired to obtain a continuous record of the dissolved oxygen content of the water under observation. The probe method may be used as a substitute for the modified Winkler procedure in BOD determinations as well.

The following method is adapted from: US EPA. 1998. Understanding Environmental Methods: Analytical Methods, Environmental Analysis, and Data Evaluation: EPA Method 360.1 (Approved for NPDES, Issued 1971); TITLE: Oxygen, Dissolved (Membrane Electrode).© Genium Publishing Corporation 1996, Schenectady, NY 12304.

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- No specific probe or accessory is especially recommended as superior. However, probes which have been evaluated or are in use and found to be reliable are the Weston & Stack DO Analyzer Model 30, the Yellow Springs Instrument (YSI) Model 54, and the Beckman Fieldlab Oxygen Analyzer.
- Follow manufacturer instructions for calibration and calculations. Manufacturer's specification claim 0.1 mg/L repeatability with ±1% accuracy.

The most common instrumental probes for determination of dissolved oxygen in water are dependent upon electrochemical reactions. Under steady-state conditions, the current or potential can be correlated with DO concentrations. Interfacial dynamics at the probe-sample interface are a factor in probe response and a significant degree of interfacial turbulence is necessary. For precision performance, turbulence should be constant. Dissolved organic materials are not known to interfere in the output from dissolved oxygen probes. However, dissolved inorganic salts are a factor in the performance of dissolved oxygen probes. Probes with membranes respond to the partial pressure of oxygen which in turn is a function of dissolved inorganic salts. Conversion factors for seawater and brackish waters may be calculated from dissolved oxygen saturation versus salinity data. Conversion factors for specific inorganic salts may be developed experimentally. Broad variations in the kinds and concentrations of salts in samples can make the use of a membrane probe difficult. Reactive compounds can interfere with the output or the performance of dissolved oxygen probes. Reactive gases which pass through the membrane probes may interfere. For example, chlorine will depolarize the cathode and cause a high probe-output. Long-term exposures to chlorine will coat the anode with the chloride of the anode metal and eventually desensitize the probe. Alkaline samples in which free chlorine does not exist will not interfere. Hydrogen sulfide can interfere with membrane probes by coating of the anode with sulfide. Again, manufacturer recommendations on probe utilization should be consulted.

PERFORMANCE

Dissolved oxygen probes are temperature sensitive and temperature compensation is normally provided by the manufacturer.

6.3 Biological Oxidation Demand (BOD)

Biological oxygen demand (BOD) is a measurement of oxygen consumption by water or wastewater microorganisms that metabolize organic materials. The oxygen "uptake rate" is measured, indicating the amount of waste in the sample and the potential for oxygen depletion in receiving waters.

BOD is an important consideration when substantial sewage is discharged into the marine environment or is suspected of impacting streams or lakes at Antarctic Stations. BOD can be measured in the waste stream before discharge to judge the effectiveness of treatment as well as in the receiving waters after discharge to detect any reductions in oxygen that might be of concern. BOD must be measured on or near the site of collection. The method involves substantial time, equipment and effort to prepare the reagents and standards. It is recommended that BOD and COD only be conducted where there is a documented concern over oxygen levels in waters that receive station discharges. Therefore, a first approach is to determine if the water bodies of concern are experiencing depressed concentrations of oxygen. Most wastewaters contain more oxygen-demanding materials than the amount of DO available in air-saturated water. Therefore, it is necessary to dilute the sample before incubation to bring the oxygen demand and supply into appropriate balance.

The following methods are adapted from: 18th Edition Standard Methods for the Examination of Water and Wastewater; Method 5210 Biochemical Oxygen Demand (BOD); Greenberg, A.E., Clesceri, L.S., and A.D. Eaton (Eds.), APHA: Washington, 1992.

"Apparatus, Materials, and Reagents - Biological Oxidation Demand"

- **Incubation bottles, 250- to 300-mL capacity**. Clean bottles with a detergent, rinse thoroughly, and drain before use. As a precaution against drawing air into the dilution bottle during incubation, use a water-seal. Obtain satisfactory water seals by inverting bottles in a water bath or by adding water to the flared mouth of special BOD bottles. Place a paper or plastic cup or foil cap over flared mouth of bottle to reduce evaporation of the water seal during incubation.
- **Air incubator or water bath, thermostatically controlled at 20 ± 1ºC**. Exclude all light to prevent possibility of photosynthetic production of DO.
- **Phosphate buffer solution**. Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7\ H_2O$, and 1.7 g NH_4Cl in about 500 mL distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment. Discard reagent (or any of the following reagents) if there is a sign of biological growth in the stock bottle.
- **Magnesium sulfate solution**. Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 L.
- **Calcium chloride solution**. Dissolve 27.5 g $CaCl_2$, in distilled water and dilute to 1 L.
- **Ferric chloride solution**. Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 L.
- **Acid and alkali solutions,** 1N, for neutralization of caustic or acidic waste samples.
  1) **Acid**- Slowly and while stirring, add 28 mL conc sulfuric acid to distilled water. Dilute to 1 L.
  2) **Alkali**- Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1 L.

- **Sodium sulfite solution**. Dissolve 1.575 g Na_2SO_3 in 1000 mL distilled water. This solution is not stable; prepare daily.
- **Nitrification inhibitor**, 2-chloro-6-(trichloro methyl) pyridine.
- **Glucose-glutamic acid solution**. Dry reagent-grade glucose and reagent grade glutamic acid at 103ºC for 1h. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 L. Prepare fresh immediately before use.
- **Ammonium chloride solution**. Dissolve 1.15 g NH_4Cl in about 500 mL distilled water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

6.3.1 Sample Preparation

In general, sample preparation consists of filling an airtight bottle of known volume with water that has been appropriately diluted with "dilution water" until it overflows, capping it, and incubating it at the specified temperature for 5 days. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between the initial and final DO. Because the initial DO is determined immediately after the dilution is made, all oxygen uptake, including that occurring during the first 15 minutes, is included in the BOD measurement. Samples for BOD analysis may degrade significantly during storage between collection and analysis resulting in low BOD values. Reductions in BOD can be minimized by promptly analyzing the sample or by cooling it to near-freezing temperature for storage. However, even at low temperature, holding times must be kept to a minimum and it is recommended that BOD analyses begin within 48 h and preferably within 24 h. Chilled samples should be warmed to 20ºC before analysis.

6.3.2 Sample Processing

6.3.2.1 Preparation of Dilution Water

- Place the desired volume of water in a suitable bottle and add 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solutions.
- Seed the dilution water with an appropriate population of microorganims (see 6.3.2.2).
- Test and store the dilution water.
- Before use, bring the dilution water temperature to 20ºC.
- Saturate with DO by shaking a partially filled bottle or by aerating with organic-free filtered air. Alternatively, store the water in cotton-plugged bottles long enough for the water to become saturated with DO.

Protect water quality by using clean glassware, tubing, and bottles.

6.3.2.2 Dilution Water Check

A dilution water check is used to confirm the quality of the dilution water. If the oxygen depletion of the dilution water exceeds 0.2 mg/L, an alternative source of water is needed. Alternatively, if nitrification inhibition is used, store the dilution water, seeded as prescribed below, in a darkened room at room temperature until the oxygen uptake is sufficiently reduced to

meet the dilution-water check criteria.

- Check the quality of the stored dilution water in use, but do not add seed to dilution water stored for quality improvement.
- Storage is not recommended when BODs are to be determined without nitrification inhibition because nitrifying organisms may develop during storage.
- Check stored dilution water to determine whether sufficient ammonia remains after storage. If not, add ammonium chloride solution to provide a total of 0.45 mg ammonia/L as nitrogen.
- If dilution water has not been stored for quality improvement, add sufficient seeding material to produce a DO uptake of 0.05 to 0.1 mg/L in 5 days at 20ºC.
- Incubate a BOD bottle full of dilution water for 5 d at 20ºC. Determine initial and final DO.

The DO uptake in 5 days at 20ºC should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L.

Because the BOD test is a bioassay, its results can be influenced greatly by the presence of toxicants or by use of a poor seeding material. Distilled waters are frequently contaminated with copper and some sewage bacterial seed populations are relatively inactive. Low results are always obtained with such seeds and waters. Periodically check the dilution water quality, seed effectiveness, and analytical technique by making BOD measurements on pure organic compounds and samples with known additions. In general, for BOD determinations not requiring an adapted seed, use a mixture of 150 mg glucose/L and 150 mg glutamic acid/L as a "standard" check solution. Glucose has an exceptionally high and variable oxidation rate but when it is used with glutamic acid, the oxidation rate is stabilized and is similar to that obtained with many municipal wastes. Alternatively, if a particular wastewater contains an identifiable major constituent that contributes to the BOD, use this compound in place of the glucose-glutamic acid. Determine the 5-days 20ºC BOD of a 2% dilution of the glucose/glutamic acid standard check solution using the standard techniques.

6.3.2.3 Seeding Material

It is necessary that the population of microorganisms be capable of oxidizing biodegradable organic matter in the sample. For such wastes, the dilution water is seeded by adding a population of microorganisms. The preferred seed is effluent from the treatment system processing the waste. Some samples may also contain materials not degraded at normal rates by the microorganisms in wastewater. Such samples with an adapted microbial population are often obtained from undisinfected effluent from waste being treated by biological processes. In the absence of such a facility, seed must be obtained from the receiving water below the point of discharge. When such seed sources are not available, develop adapted seed in the laboratory by continuously aerating a sample of settled wastewater and adding small daily increments of waste. Optionally, use a commercial seed preparation to obtain the initial microbial population. Determine the existence of a satisfactory population by testing the performance of the seed in BOD tests on the sample. BOD values that increase with time of adaptation to a steady high

value indicate successful seed adaptation.

Determine the BOD of the seeding material as for any other sample. This is the seed control. From the value of the seed control and a knowledge of the seeding material dilution (in the dilution water) determine seed DO uptake. Ideally, make dilutions of the seed such that the largest quantity results in at least a 50% depletion in DO. A plot of DO depletion, in milligrams per liter, versus milliliters of seed should be a straight line and the slope indicates DO depletion per milliliter of seed. The DO-axis intercept is oxygen depletion caused by the dilution water and should be less than 0.1 mg/L (~ 4h). To determine a sample DO uptake, subtract seed DO uptake from total DO uptake. The DO uptake of seeded dilution water should be between 0.6 and 1.0 mg/L.

Samples containing caustic alkalinity or acidity should be neutralized to ~pH 6.5 to 7.5 with a solution of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) of such strength that the quantity of reagent does not dilute the sample by more than 0.5%. The pH of seeded dilution water should not be affected by the lowest sample dilution.

When using graduated cylinders to prepare dilutions, and when seeding is necessary, add seed either directly to dilution water or to individual cylinders before dilution. Seeding of individual cylinders avoids a declining ratio of seed to sample as increasing dilutions are made. When dilutions are prepared directly in BOD bottles and when seeding is necessary, add seed directly to dilution water or directly to the BOD bottles. If the azide modification of the titrimetric iodometric method for DO measurement is used:

- Carefully siphon dilution water, seeded if necessary, into a 1- to 2-L-capacity graduated cylinder.
- Fill cylinder half full without entraining air.
- Add the desired quantity of carefully mixed sample and dilute to appropriate level with dilution water.
- Mix well with a plunger-type mixing rod; avoid entraining air.
- Siphon the mixed dilution into two BOD bottles.
- Determine the initial DO on one of these bottles.
- Stopper the second bottle, tightly water-seal, and incubate for 5 days at 20ºC.
- If the membrane electrode method is used for DO measurement, siphon the dilution mixture into one BOD bottle.
- Determine the initial DO on this bottle and replace any displaced contents with sample dilution to fill the bottle.
- Stopper tightly, water-seal, and incubate for 5 days at 20ºC.

To prepare dilutions in BOD bottles:

- Use a wide tip volumetric pipet.
- Add the desired sample volume to seeded BOD bottles of known capacity.
- Add appropriate amounts of seed material to the individual BOD bottles or to the dilution water.
- Fill bottles with enough dilution water, seeded if necessary, so that insertion of the stopper will displace all air, leaving no bubbles.

- For dilutions greater than 1:100 make a primary dilution in a graduated cylinder before making the final dilution in the bottle.
- When using titrimetric iodometric methods for DO measurement, prepare two bottles at each dilution.
- Determine initial DO on one bottle. Stopper the second bottle tightly, water seal, and incubate for 5 days at 20ºC.

6.3.2.4 Use of DO Probe or Sensor

If the membrane electrode method is used for DO measurement, prepare only 1 BOD bottle for each dilution. Determine initial DO on this bottle and replace any displaced contents with dilution water to fill the bottle. Stopper tightly, water-seal, and incubate for 5 days at 20ºC. Rinse the DO electrode between determinations to prevent cross contamination of samples.

If the sample contains materials that react rapidly with DO, determine initial DO immediately after filling the BOD bottle with diluted sample. If rapid initial DO uptake is insignificant, the time period between preparing dilution and measuring initial DO is not critical Use the azide modification of the iodometric method or the membrane electrode method to determine the initial DO on all sample dilutions, dilution water blanks, and where appropriate seed controls. Use a dilution water blank as a check on the quality of unseeded dilution water and cleanliness of incubation bottles. Together with each batch of samples incubate a bottle of unseeded dilution water. Determine initial and final DO. The DO uptake should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L.

Incubate BOD bottles containing the desired dilutions, seed controls, dilution water blanks, and glucose-glutamic acid checks at 20ºC $\pm$ 1ºC. Water-seal all bottles. After 5 days incubation, determine the DO in sample dilutions, blanks, and checks.

6.3.3 Calculations

When dilution water is not seeded:

$$BOD_5, mg/L = (D_1 - D_2)/P$$

When dilution water is seeded:

$$BOD_5, mg/L = [(D_1 — D_2) — (B_1 — B_2)\ f]/P$$

where:

BOD_5 = BOD after 5 days
D_1 = DO of diluted sample immediately after preparation in mg/L,
D_2 = DO of diluted sample after 5 days incubation at 20ºC in mg/L,
P = decimal volumetric fraction of sample used,
B_1 = DO of seed control before incubation in mg/L,
B_2 = DO of seed control after incubation in mg/L and
f = ratio of seed in diluted sample to seed in seed control

(= % seed in diluted sample)/(% seed in seed control).

If seed material is added directly to sample or to seed control bottles:

f = (volume of seed in diluted sample)/(volume of seed in seed control)

Report results as $CBOD_5$ if nitrification is inhibited.

If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L, and there is no evidence of toxicity at higher sample concentrations or the existence of an obvious anomaly, average the results.

There is no measurement for establishing bias of the BOD procedure. The glucose-glutamic acid check is intended to be a reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique. Single-laboratory tests using a 300-mg/L mixed glucose-glutamic acid solution provided the following results:

Number of months: 14
Number of triplicates: 421
Average monthly recovery: 204 mg /L
Average monthly standard deviation: 10.4 mg / L

In a series of interlaboratory studies, each involving 2 to 112 laboratories (and as many analysts and seed sources), 5-day BOD measurements were made on synthetic water samples containing a 1:1 mixture of glucose and glutamic acid in the total concentration range of 3.3 to 231 mg/L. The regression equations for mean value, X, and standard deviation, S, from these studies were:

X = 0.658 (added level, mg/L) + 0.280 mg/L
S = 0.100 (added level, mg/L) + 0.547 mg/L

For the 300 mg/L mixed primary standard, the average 5-day BOD would be 198 mg/L with a standard deviation of 30.5 mg/L.

PERFORMANCE

Because of many factors affecting BOD tests in multilaboratory studies and the resulting variability in test results, one standard deviation, as determined by interlaboratory tests, is recommended as a control limit for individual laboratories. Alternatively, for each laboratory, establish the control limits by performing a minimum of 25 glucose-glutamic acid checks over a period of several weeks or months and calculate the mean and standard deviation. Use the mean $\pm$ 3 standard deviations as the control limit for future glucose-glutamic acid checks. Compare calculated control limits to the single laboratory tests presented above and to the interlaboratory results. If control limits are outside the range of 198 ± 30.5, reevaluate the control limits and investigate the source of the problem. If the measured BOD for a glucose-glutamic acid check is outside the accepted control limit range, reject the tests made with that

seed and dilution water.

The working range is equal to the difference between the maximum initial DO (7 to 9 mg/L) and minimum DO residual of mg/L multiplied by the dilution factor. A lower detection limit of 2 mg/L is established by the requirement for a minimum DO depletion of 2 mg/L.

6.4 Chemical Oxidation Demand (COD)

Chemical oxygen demand (COD) is a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. For samples from a specific source, COD can be related empirically to BOD, organic carbon, or organic matter. The test is useful for monitoring and control after a correlation has been established. The dichromate reflux method is preferred over procedures using other oxidants because of superior oxidizing ability, applicability to a wide variety of samples, and ease of manipulation. Oxidation of most organic compounds is 95 to 100% of the theoretical value. Pyridine and related compounds resist oxidation and volatile organic compounds are oxidized only to the extent that they remain in contact with the oxidant. Ammonia, present either in the waste or liberated from nitrogen-containing organic matter is not oxidized in the absence of significant concentrations of free chloride ions.

The following method is adapted from: 18th Edition Standard Methods for the Examination of Water and Wastewater; Method 5220: Chemical Oxygen Demand (COD); A.E. Greenberg, L.S. Clesceri, and A.D. Eaton (Eds.), APHA: Washington, 1992.

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- **Reflux apparatus**, consisting of 500- or 250-mL Erlenmeyer flasks with ground-glass 24/40 neck and 300-mm jacket Liebig, West, or equivalent condensers with 24/40 ground-glass joint.
- **A hot plate** having sufficient power to produce at least 1.4 W/cm^2 of heating surface or equivalent.
- **Standard potassium dichromate solution**, 0.0417M: Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 103ºC for 2 h, in distilled water and dilute to 1000 mL.
- **Sulfuric acid reagent**. Add Ag_2SO_4, reagent or technical grade, crystals or powder, to conc H_2SO_4 at the rate of 5.5 g Ag_2SO_4/kg H_2SO_4. Let stand 1 to 2 d to dissolve Ag_2SO_4.
- **Ferroin indicator solution**. Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $FeSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 mL. This indicator solution may be purchased already prepared.
- **Standard ferrous ammonium sulfate** (FAS) titrant, approximately 0.25M: Dissolve 98 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 mL conc H_2SO_4, cool, and dilute to 1000 mL. Standardize this solution daily against standard $K_2Cr_2O_7$ solution as follows:
  - Dilute 10.0 mL standard $K_2Cr_2O_7$ to about 100 mL. Add 30 mL conc H_2SO_4 and cool.
  - Titrate with FAS titrant using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

- Molarity of FAS solution = 0.25 x (Volume 0.0417M $K_2Cr_2O_7$ solution titrated, mL)/(Volume FAS used in titration, mL)

- **Mercuric sulfate**, $HgSO_4$, crystals or powder.
- **Sulfamic acid**. Required only if the interference of nitrites is to be eliminated. Add 10 mg sulfamic acid for each mg of NO_2^--N mL sample volume.
- **Potassium hydrogen phthalate (KHP) standard**. Lightly crush and then dry potassium hydrogen phthalate ($HOOCC_6H_4COOK$) to constant weight at 120ºC. Dissolve 425 mg in distilled water and dilute to 1000 mL. KHP has a theoretical COD of 1.176 mg O_2/mg and this solution has a theoretical COD of 500 µg O_2/mL. This solution is stable when refrigerated for up to 3 months in the absence of visible biological growth.

The open reflux method is suitable for a wide range of waters where a large sample size is available. The closed reflux methods are more economical in the use of metallic salt reagents, but require homogenization of samples containing suspended solids to obtain reproducible results. Ampules and culture tubes with premeasured reagents are commercially available. Follow the instructions furnished by the manufacturer.

Volatile straight-chain aliphatic compounds are not oxidized to any appreciable extent. This failure occurs partly because volatile organics are present in the vapor space and do not come in contact with the oxidizing liquid. Straight-chain aliphatic compounds are oxidized more effectively when silver sulfate (Ag_2SO_4) is added as a catalyst. However Ag_2SO_4 reacts with chloride, bromide, and iodide to produce precipitates that are only partially oxidized. The difficulties caused by the presence of halides can be overcome largely, though not completely, by complexing them with mercuric sulfate ($HgSO_4$). Although 1 g $HgSO_4$ is specified for a 50 mL sample, a lesser amount may be used where sample chloride concentration is known to be less than 2000 mg/L as long as a 10:1 ratio of $HgSO_4$:Cl^- is maintained. Do not use the test for samples containing more than 2000 mg Cl^-/L. Techniques designed to measure COD in saline waters are available.

Reduced inorganic species such as ferrous iron, sulfide, manganous manganese, etc., are oxidized quantitatively under the test conditions. For samples containing significant levels of these species, stoichiometric oxidation can be assumed from the known initial concentration of the interfering species and corrections can be made to the COD value obtained.

Collect samples in glass or plastic bottles. Test unstable samples without delay. If delay before analysis is unavoidable, preserve samples by acidification to pH <2 using conc. H_2SO_4. Preferably, acidify any sample that cannot be analyzed the same day it is collected. Blend samples containing settable solids with a homogenizer to ensure representative sampling. Make preliminary dilutions for wastes containing a high COD to reduce the error inherent in measuring small sample volumes.

As for BOD, COD requires extensive equipment, reagent preparation, and analyst time and may not be applicable to remote Antarctic sites without substantial infrastructure. The reagents utilized are very caustic and require suitable facilities to handle safely.

6.4.1 Open Reflux Method

Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable organic matter is calculated in terms of oxygen equivalents. Keep ratios of reagent weights, volumes, and strengths constant when sample volumes other than 50 mL are used. The standard 2-h reflux time may be reduced if it has been shown that a shorter period yields the same results.

6.4.1.1 Sample Preparation and Processing

Treatment of samples with COD of >50 mg O_2/L requires placement of a 50 mL sample (for samples with COD of >900 mg O_2/L. use smaller sample portion diluted to 50 mL) in a 500-mL refluxing flask. Once proportioned into the flask:

- Add 1 g $HgSO_4$, several glass beads, and very slowly 5.0 mL of sulfuric acid reagent with mixing to dissolve the $HgSO_4$.
- Cool while mixing to avoid the possible loss of volatile materials.
- Add 25.0 mL 0.0417M $K_2Cr_2O_7$ solution and mix.
- Attach the flask to the condenser and turn on cooling water.
- Add the remaining sulfuric acid reagent (70 mL) through the open end of the condenser.
- Continue swirling and mixing while adding the sulfuric acid reagent.

[CAUTION: Mix the reflux mixture thoroughly before applying heat to prevent local heating of the flask bottom causing a possible blowout of the flask's contents.]

Cover the open end of condenser with a small beaker to prevent foreign material from entering the refluxing mixture and reflux for 2 h. Then,

- Cool and wash down the condenser with distilled water.
- Disconnect the reflux condenser and dilute the mixture to about twice its volume with distilled water.
- Cool to room temperature and titrate the excess $K_2Cr_2O_7$ with FAS, using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.
- Although the quantity of ferroin indicator is not critical, use the same volume for all titrations.
- Take as the end point of the titration the first sharp color change from blue-green to reddish brown although the blue-green may reappear.
- In the same manner, reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of sample.

For low-COD samples follow the above procedure with two exceptions: (i) use standard 0.00417M $K_2Cr_2O_7$. and (ii) titrate with 0.025M FAS. Exercise extreme care with this procedure because even a trace of organic matter on the glassware or from the atmosphere may cause

errors.

If a further increase in sensitivity is required, concentrate a larger volume of sample before digesting under reflux.

- Add all reagents to a sample larger than 50 mL and reduce total volume to 50 mL by boiling in the refluxing flask open to the atmosphere without the condenser attached.
- Compute the amount of $HgSO_4$ to be added (before concentration) on the basis of a weight ratio of 10:1, $HgSO_4$:Cl^-, using the amount of Cl^- present in the original volume of sample.
- Carry a blank reagent through the same procedure.

This technique has the advantage of concentrating the sample without significant losses of easily digested volatile materials. Hard-to-digest volatile materials such as volatile acids are lost but an improvement is gained over ordinary evaporative concentration methods. Evaluate the technique and quality of reagents by conducting the test on a standard potassium hydrogen phthalate solution.

6.4.1.2 Calculations

COD is calculated as mg O_2/L = [(A-B) x M x 8000]/mL sample:

where:

A = mL FAS used for blank.
B = mL FAS used for sample, and
M = molarity of FAS.

PERFORMANCE

A set of synthetic samples containing potassium hydrogen phthalate and NaCl was tested by 74 laboratories. At a COD of 200 mg O_2/L in the absence of chloride, the standard deviation was ± 13 mg/L (coefficient of variation, 6.5%). At COD of 160 mg O_2/L and 100 mg Cl^-/L, the standard deviation was ± 14 mg/L (coefficient of variation, 10.8%).

6.4.2 Closed Reflux Method - #1 - Sample Preparation and Processing

To prepare a sample for processing:

- Wash culture tubes and caps with 20% H_2SO_4 before the first use to prevent contamination.
- Refer to the summary for proper sample and reagent volumes.
- Place the sample in a culture tube or ampule and add the digestion solution.
- Carefully run the sulfuric acid reagent down the inside of the vessel so an acid layer is formed under the sample-digestion solution layer.
- Tightly cap the tubes or seal the ampules, and invert each several times to mix

completely.

CAUTION**:** Wear face shield and protect hands from the heat produced when the contents of vessels are mixed. Mix thoroughly before applying heat to prevent local heating of vessel bottom causing a possible explosive reaction.

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- **Digestion vessels**. Preferably use borosilicate culture tubes, 16- x 100-mm, 20- x 150-mm. or 25- x 150-mm. with Teflon-lined screw caps. Alternatively, use borosilicate ampules, 10-mL capacity, 19- to 20-mm diameter.
- **Heating block**, cast aluminum, 45 to 50 mm deep. with holes sized for close fit of culture tubes or ampules.
- **Block heater or oven**, to operate at 150 $\pm$ 2ºC. NOTE: Severe damage of most culture tube closures from oven digestion introduces a potential source of contamination and increases the probability of leakage. Use an oven for culture-tube digestion only when it has been determined that 2 h exposure at 150ºC will not damage the caps.
- **Ampule sealer**. Use only a mechanical sealer to insure strong, consistent seals.
- **Standard potassium dichromate digestion solution**, 0.0167M: Add to about 500 mL distilled water 4.913 g $K_2Cr_2O_7$, primary standard grade, previously dried at 103ºC for 2 h, 167 mL conc H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 mL.
- **Sulfuric acid reagent**. See Section above.
- **Ferroin indicator solution**. See Section above.
- **Standard ferrous ammonium sulfate titrant** (FAS), approximately 0.10M: Dissolve 39.2 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 mL conc H_2SO_4. cool, and dilute to 1000 mL. Standardize solution daily against standard $K_2Cr_2O_7$ digestion solution as follows. Add reagents according to the table below to a culture tube containing the correct volume of distilled water substituted for sample.

Sample and Reagent Quantities for Various Digestion Vessels

Digestion Vessel	Sample mL	Digestion Solution mL	Sulfuric Acid Reagent mL	Total Final Volume mL
Culture tubes:				
16 x 100 mm	2.5	1.5	3.5	7.5
20 x 150 mm	5.0	3.0	7.0	15.0
25 x 150 mm	10.0	6.0	14.0	30.0
Standard 10-mL ampules	2.5	1.5	3.5	7.5

- Cool tube to room temperature and add 0.05 to 0.10 mL (1 to 2 drops) ferroin indicator and titrate with FAS titrant.

  Molarity of FAS solution =

[(Volume 0.0167M $K_2Cr_2O_7$ solution titrated, mL)/(Volume FAS used in titration, mL)] x 0.10

- **Sulfamic acid**. See Section above.
- **Potassium hydrogen phthalate standard**. See Section above.

- Place the tubes or ampules in a block digester or oven, preheat to 150ºC, and reflux for 2 h.

- Cool to room temperature and place the vessels in test tube rack.
- Remove the culture tube caps and add a small TFE-covered magnetic stirring bar.
- Transfer the contents to a larger container for titrating if ampules are used.
- Add 0.05 to 0.10 mL (i.e., 2 drops) ferroin indicator and stir rapidly on a magnetic stirrer while titrating with 0.10 M FAS.
- The end point is a sharp color change from blue-green to reddish brown, although the blue-green may reappear within minutes.
- In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample.

6.4.2.1 Calculations

COD is calculated as as mg O_2/L = [(A-B) x M x 8000]/mL sample:

where:

A = mL FAS used for blank.
B = mL FAS used for sample. and
M = molarity of FAS.

PERFORMANCE

Sixty synthetic samples containing potassium hydrogen phthalate and NaCl were tested by six laboratories. At an average COD of 195 mg O_2/L in the absence of chloride, the standard deviation was ± 11 mg O_2/L (coefficient of variation, 5.6%). At an average COD of 208mg O_2/L and 100 mg Cl^-/L, the standard deviation was ± 10 mg O_2/L (coefficient of variation, 4.8%).

6.4.3 Closed Reflux Method - #2 - Sample Preparation and Processing

To prepare a sample for processing:

- Measure a suitable volume of sample and reagents into a tube or ampule as indicated above.
- Prepare, digest, and cool samples, blanks, and one or more standards as directed above.
- To measure dichromate reduction, invert the cooled samples, blanks, and standards several times and allow the solids to settle before measuring absorbance.
- Dislodge solids that adhere to the container's walls by gentle tapping and settling.
- Insert the unopened tube or ampule through the access door into the light path of spectrophotometer set at 600 nm.
- Read absorbance and compare the calibration curve.
- Use optically matched culture tubes or ampules for greater sensitivity.

- Discard any scratched or blemished glassware.

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- See Section above.
- **Spectrophotometer**, for use at 600 nm with access opening adapter for ampule or 16-, 20- or 25-mm tubes.
- **Digestion solution**. Add to about 500 mL distilled water 10.216 g $K_2Cr_2O_7$, primary standard grade, previously dried at 103ºC for 2 h, 167 mL conc H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature and dilute to 1000 mL.
- **Sulfamic acid reagent**. See above.
- **Sulfuric acid**. See Section above.
- **Potassium hydrogen phthalate standard**. See Section above.

- Prepare at least five standards from potassium hydrogen phthalate solution with COD equivalents from 20 to 900 mg O_2/L.
- Make up the volume with distilled water and use the same reagent volumes, tube, or ampule size, and digestion procedure as for samples.
- Prepare calibration curves for each new lot of tubes or ampules.

6.4.3.1 Calculations

COD is calculated as as mg O_2/L = (mg O_2 in final volume x 1000)/mL sample.

PERFORMANCE

Forty-eight synthetic samples containing potassium hydrogen phthalate and NaC1 were tested by five laboratories. At an average COD of 193 mg O_2/L in the absence of chloride, the standard deviation was ± 17 mg O_2/L (coefficient of variation 8.7%). At an average COD of 212 mg O_2/L and 100 mg Cl^-/L, the standard deviation was ± 20 mg O_2/L (coefficient of variation, 9.6%).

6.5 Acidity

This method is applicable to rain, surface, and other waters of pH less than 8.3. Acidity is a measure of the concentration of strong and weak acids that react with hydroxyl ions. This includes any dissolved gases that are present. The range of this method depends on the volume of sample titrated and upon the precision with which the increments of titrant can be measured. If only 10 mL of sample is available for analysis, it is necessary to use a 50 µL syringe for dispensing the titrant in order to achieve a precision of less than 10 µeq/L. Samples are titrated with 0.02 N carbonate-free NaOH solution. The end point is determined with a pH meter. Results are reported as microequivalents (µeq) per liter. The sample container must be filled completely, sealed and stored at 4°C. Care must be taken to minimize exposure of the sample to the atmosphere. Open the sample container immediately before analysis. Analysis should be performed as soon as possible after collection.

The following method is adapted from: US EPA. 1998. Understanding Environmental Methods: Analytical Methods, Environmental Analysis, and Data Evaluation: EPA Method 305.2 (Test Method, December 1982); TITLE: Acidity (Titrimetric).© Genium Publishing Corporation 1996, Schenectady, NY 12304.

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- **pH meter and electrode(s)**
- **Micro buret or micro syringes.**
- **Teflon or glass magnetic stirring bar.**
- **Magnetic stirrer.**
- **Beakers or flasks.**
- **Standard sodium hydroxide solution**, 1 N: Dissolve 40 g NaOH in 250 mL distilled water. Cool and dilute to 1 liter with CO_2 free distilled water. Store in a polyolefin bottle and fitted with a soda lime tube or tight cap to protect from atmospheric CO_2.
- **Standard sodium hydroxide titrant**, 0.02 N: Dilute 20.0 mL of 1 N NaOH with CO_2-free distilled water to 1 liter. Store in rubber stoppered bottle. Protect from atmospheric CO_2 by using a soda lime tube. Standardize against an 0.02 N potassium hydrogen phthalate solution prepared by dissolving 4.085 g of anhydrous $KHC_8H_4O_4$ in CO_2-free distilled water and diluted to 1 L.

6.5.1 Sample Preparation and Processing

Samples with an initial pH between 4.3 and 8.3 are subject to error due to the loss or gain of dissolved gases during sampling, storage and analysis. To prepare and process a sample:

- Pipet an appropriate aliquot of sample into a beaker or flask containing a small Teflon or glass stirring bar.
- Use extreme care to minimize the sample surface disturbance.
- Immerse the pH electrode(s) into the sample and stir at a rate that does not cause sample surface disturbance.
- Titrate with 0.02 N NaOH to pH 8.3. Titration should be made as quickly as possible to prevent absorption of atmospheric CO_2.
- Record the volume of the titrant.

6.5.2 Calculations

$$\text{Normality of NaOH, N(B)} = \frac{\text{mL(K) x 0.020}}{\text{mL(B)}}$$

Acidity is calculated as $\mu eq/L = (mL(B)/mL(s)) \times N(B) \times 10^6$ where

$\mu eq/L$ = microequivalents per liter
mL(K) = mL of $KHC_8H_4O_4$
mL(B) = mL of NaOH titrant
mL(s) = mL of sample

N(B) = normality of titrant

Precision and accuracy data are not available.

6.6 pH

This method is applicable to marine waters, freshwaters, and wastewaters. The pH of a sample is determined electrometrically using either a glass electrode in combination with a reference potential, a combination electrode or stainless steel probe with silicon chip sensor and temperature sensor. Samples should be analyzed as soon as possible, preferably in the field at the time of sampling.

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- **pH Meter**-laboratory or field model. A wide variety of instruments are commercially available with various specifications and optional equipment.
- **Glass electrode**.
- **Reference electrode**-a calomel, silver-silver chloride or other reference electrode of constant potential may be used. NOTE: Combination electrodes incorporating both measuring and reference functions are convenient to use and are available with solid, gel type filling materials that require minimal maintenance.
- **Magnetic stirrer and Teflon-coated stirring bar**.
- **Thermometer or temperature sensor for automatic compensation**.
- **Primary standard buffer salts** are available from the National Institute of Standards and Tests (NIST) and should be used in situations where extreme accuracy is necessary. Preparation of reference solutions from these salts require some special precautions and handling, such as low conductivity dilution water, drying ovens, and carbon dioxide free purge gas. These solutions should be replaced at least once each month.
- **Secondary standard buffers** may be prepared from NIST salts or purchased as a solution from commercial vendors. Use of these commercially available solutions, that have been validated by comparison to NIST standards, are recommended for routine use.
- Because of the wide variety of pH meters and accessories, detailed operating procedures cannot be incorporated into this method. Each analyst must be acquainted with the operation of each system and familiar with all instrument functions. Special attention to care of the electrodes is recommended.
- Each instrument/electrode system must be calibrated at a minimum of two points that bracket the expected pH of the samples and are approximately three pH units or more apart. Various instrument designs may involve use of a "balance" or "standardize" dial and/or a slope adjustment as outlined in the manufacturer's instructions. Repeat adjustments on successive portions of the two buffer solutions as outlined in procedure 8.2 until readings are within 0.05 pH units of the buffer solution value.

Glass electrodes, in general, are not subject to solution interferences from color, turbidity, colloidal matter, oxidants, reductants or high salinity. Coatings of oily material or particulate matter can impair electrode response. These coatings can usually be removed by gentle wiping or detergent washing, followed by distilled water rinsing. An additional treatment with hydrochloric acid may be necessary to remove any remaining film. Temperature effects on the electrometric measurement of pH arise from two sources. The first is caused by a change in electrode output at

various temperatures. This interference can be controlled with instruments having temperature compensation or by calibrating the electrode-instrument system at the temperature of the samples. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled. It should therefore be noted by reporting both the pH and temperature at the time of analysis.

The following method is adapted from: US EPA Methods for Chemical Analysis of Water and Wastes. Environmental Monitoring Systems Laboratory - Cincinnati ((EMSLCI), EPA-600/4-79-020, Revised March 1983 and 1979.

6.6.1 Sample Preparation and Processing

To prepare a sample for analysis:

- Standardize the meter and electrode system as instructed by the manufacturer.
- Place the sample or buffer solution in a clean glass beaker using a sufficient volume to cover the sensing elements of the electrodes and to give adequate clearance for the magnetic stirring bar.
- If field measurements are being made, the electrodes may be immersed directly in the sample stream to an adequate depth and moved in a manner to insure sufficient sample movement across the electrode sensing element as indicated by drift free (< 0.1 pH) readings.
- If the sample temperature differs by more than 2ºC from the buffer solution, the measured pH values must be corrected.
- Instruments are equipped with automatic or manual compensators that electronically adjust for temperature differences. Refer to the manufacturer's instructions.
- After rinsing and gently wiping the electrodes, if necessary, immerse them into the sample beaker or sample stream and stir at a constant rate to provide homogeneity and suspension of solids.
- The rate of stirring should minimize the air transfer rate at the air-water interface of the sample.

Note and record sample pH and temperature. Repeat the measurement on successive volumes of sample until values differ by less than 0.1 pH units. Two or three volume changes are usually sufficient.

6.6.2 Calculations

pH meters read directly in pH units. Report pH to the nearest 0.1 unit and temperature to the nearest degree C.

PERFORMANCE

Forty-four analysts in twenty laboratories analyzed six synthetic water samples containing exact increments of hydrogen hydroxyl ions, with the following results:

Accuracy as pH Units	Standard Deviation Bias, Bias, pH Units,	% pH Units
3.5	0.10	-0.29	-0.01
3.5	0.11	-0.00	-------
7.1	0.20	+1.01	+0.07
7.2	0.18	-0.03	-0.002
8.0	0.13	-0.12	-0.01
8.0	0.12	+0.16	+0.01

6.7 Conductivity

Conductivity is a measure of the ability of an aqueous solution to carry an electric current and depends on the presence of ions (e.g., total concentration, mobility, and valence) and the temperature of the measurements. Conductivity is a useful measurement to determine the degree of mineralization (commonly called total dissolved solids) in water. This information is used to determine the overall ionic effect of a water source and evaluate the potential for physiological effects on plants and animals.

The following method is adapted from 18th Edition Standard Methods for the Examination of Water and Wastewater, Method 2510: Conductivity; Greenberg, A.E., Clesceri, L.S., and A.D. Eaton (eds), APHA: Washington, 1992.

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- **Self-Contained Conductivity Instruments**. Use an instrument capable of measuring conductivity with an error not exceeding 1% or 1 μmho/cm, whichever is greater.
- **Thermometer**. Capable of being read to the nearest 0.1ºC and covering the range 23 to 27ºC.
- **Conductivity Cell:**
  1. Platinum-electrode type. Conductivity cells containing platinized electrodes are available in either pipet or immersion form. Cell choice depends on expected range of conductivity. Experimentally check instrument by comparing instrumental results with true conductivities of the KCl solutions listed in the following table:

Equivalent Conductivity, Λ, and Conductivity, *k*, of Potassium Chloride at 25.0ºC*

KCl Concentration *M or equivalent/L*	Equivalent Conductivity, *μmho-cm²/equivalent*	Conductivity, *k*, *μmho/cm*
0	149.9	
0.0001	148.9	14.9
0.0005	147.7	73.9
0.001	146.9	146.9
0.005	143.6	717.5
0.01	141.2	1 412

0.02	138.2	2 765
0.05	133.3	6 667
0.1	128.9	12 890
0.2	124.0	24 800
0.5	117.3	58 670
1	111.9	111 900

*Based on the absolute ohm, the 1968 temperature standard, and the dm^3 volume standard.[2] Values are accurate to ±0.1% or 0.1 μmho/cm, whichever is greater.

Clean new cells, not already coated and ready for use, with chromic-sulfuric acid cleaning mixture and platinize the electrodes before use. Subsequently, clean and replatinize them whenever the readings become erratic, when a sharp end point cannot be obtained, or when inspection shows that any platinum black has flaked off. To platinize, prepare a solution of 1 g chloroplatinic acid, H_2PtCl_6-$6H_2O$, and 12 mg lead acetate in 100 mL distilled water. Immerse electrodes in this solution and connect both to the negative terminal of a 1.5-V dry cell battery. Connect positive side of battery to a piece of platinum wire and dip wire into the solution. Use a current such that only a small quantity of gas is evolved. Continue electrolysis until both cell electrodes are coated with platinum black. Rinse electrodes thoroughly and when not in use keep immersed in distilled water.

2. Nonplatinum electrode type--Use conductivity cells containing electrodes constructed from durable common metals (stainless steel among others) for continuous monitoring and field studies. Calibrate such cells by comparing sample conductivity with results obtained with a laboratory instrument. Use properly designed and mated cell and instruments to minimize errors in cell constant.

- **Conductivity Water**. Any of several methods can be used to prepare reagent-grade water, e.g., distillation. The conductivity should be small compared to the value being measured.
- **Standard Potassium chloride Solution**. KCl, 0.0100*M*; Dissolve 745.6 mg anhydrous KCl in conductivity water and dilute to 1000 mL in a class A volumetric flask at 25ºC. This is the standard reference solution, which at 25ºC has a conductivity of 1412 μmho/cm. It is satisfactory for most samples when the cell has a constant between 1 and 2 cm^{-1}.

6.7.1 Sample Preparation and Processing

Determine the cell constant by rinsing the conductivity cell with at least three portions of 0.01*M* KCl solution. Adjust the temperature of a fourth portion to 25.0 ± 0.1ºC. If the conductivity meter displays resistance, measure resistance and note the temperature. Compute the cell constant, C by:

$$C(cm^{-1}) = (0.001\ 412)(R_{KCl})[1 + 0.019(t-25)]$$

where:

R_{KCl} = measured resistance, mho, and
t = observed temperature, ºC.

Conductivity meters often directly indicate conductivity. Commercial probes commonly contain a temperature sensor. With such instruments, rinse the probe three times with 0.0100*M* KCl, as above. Adjust the temperature compensation dial to 0.0191ºC. With the probe in standard KCl solution, adjust the meter to read 1412 µmho/cm. this procedure automatically adjusts the cell constant internal to the meter.

To measure conductivity, rinse the cell with one or more portions of sample. Adjust the temperature of a final portion of the sample to about 25.0ºC. Measure sample resistance or conductivity and note the temperature to ± 0.1ºC.

The temperature coefficient of most waters is only approximately the same as that of the standard KCl solution. The more the temperature of the measurement deviates from 25ºC, the greater the uncertainty in applying the temperature correction. Report all conductivities at 25.0ºC.

6.7.2 Calculations

When sample resistance is measured, conductivity at 25ºC is:

$$k = \frac{(1{,}000{,}000)(C)}{R_m[1 + 0.019(t - 25)]}$$

where:

k = conductivity, µmho/cm,
C = cell constant, cm^{-1},
R_m = measured resistance of sample, mho, and
t = temperature of measurement.

When sample conductivity is measured without internal temperature compensation conductivity is:

$$k, \mu hmo/cm = \frac{(k_m)}{1 + 0.0191(t - 25)}$$

where:

k_m = measured conductivity in units of µmho/cm at tºC, and other units are defined as above.

For instruments with automatic temperature compensation and readout directly in µmho/cm or similar units, the readout is automatically corrected to 25ºC. Report the displayed conductivity in the designated units.

For instruments giving values in SI units,

1 mS/m = 10 µmho/cm, or conversely,
1 µmho/cm = 0.1 mS/m.

PERFORMANCE

The precision of commercial conductivity meters is commonly between 0.1 and 1.0%. Reproducibility of 1 to 2% is expected after an instrument has been calibrated with such data shown in the table above.

6.8 Nitrate/Nitrite

Nitrogen is a required micronutrient for the growth of biological organisms. The introduction of nitrate into waters can cause a proliferation of biological organisms if nitrogen is naturally limiting to growth. The two most important forms of nitrogen dissolved in water are nitrate and nitrite. Bacteria are capable of reducing nitrate to nitrite, so often the total nitrogen load is measured as a combination of these two species. Other forms of nitrogen are often present such as ammonia and organic nitrogen but these forms are generally less important than nitrate. Eutrophication induced by the discharge of excess nutrients to receiving waters can influence water quality. Eutrophication is caused by the consumption of dissolved oxygen during the aerobic decomposition of the organic remains of organisms.

6.8.1 Spectrophotometric, Cadmium Reduction Method

This method can be applied to the determination of nitrite, or nitrite and nitrate combined in marine waters, freshwater, and wastewaters and is adapted from EPA Method 353.3. The applicable range of this method is 0.01 to 1.0 mg/L nitrate-nitrite nitrogen. Samples with high concentrations can be diluted to fall within the applicable range.

Apparatus, Materials, and Reagents for Nitrite/Nitrate

Reduction column: The column in Figure 6.1 was constructed from a 100 mL pipet by removing the top portion. This column may also be constructed from two pieces of tubing joined end to end. A 10 cm length of 3 mm in diameter tubing is joined to a 25 cm length of 3.5 mm inner diamter (ID) tubing or equivalent.
Spectrophotometer for use at 540 nm, providing a light path of 1 cm or longer.
Granulated cadmium: 40-60 mesh (MCB Reagents).
Copper-Cadmium: The cadmium granules (new or used) are cleaned with dilute HCl and copperized with 2% solution of copper sulfate in the following manner: 1) Wash the cadmium with dilute HCl and rinse with distilled water. The color of the cadmium should be silver. 2) Swirl 25 g cadmium in 100 mL portions of a 2% solution of copper sulfate for 5 minutes or until blue color partially fades, decant and repeat with fresh copper sulfate until a brown colloidal precipitate forms. 3) Wash the copper-cadmium with distilled water (at least 10 times) to remove all the precipitated copper. The color of the cadmium so treated should be black.
Preparation of reaction column: Insert a glass wool plug into the bottom of the reduction column and fill with distilled water. Add sufficient copper-cadmium granules to produce a column 18.5 cm in length. Maintain a level of distilled water above the copper- cadmium granules to eliminate entrapment of air. Wash the column with 200 mL of dilute ammonium chloride solution. The column is then activated by passing through the column 100 mL of a solution composed of 25 mL of a 1.0 mg/L NO_3^-N standard and 75 mL of ammonium chloride-EDTA solution. Use a flow rate between 7 and 10 mL per minute.
Ammonium chloride- EDTA solution: Dissolve 13 g ammonium chloride and 1.7 g disodium ethylenediamine tetraacetate in 900 mL of distilled water. Adjust the pH to

8.5 with conc. ammonium hydroxide and dilute to 1 liter.
Dilute ammonium chloride-EDTA solution: Dilute 300 mL of ammonium chloride-EDTA solution to 500 mL with distilled water. Color reagent: Dissolve 10 g sulfanilamide and 1 g N-(1-naphthyl)-ethylenediamine dihydrochloride in a mixture of 100 mL conc. phosphoric acid and 800 mL of distilled water and dilute to 1 liter with distilled water.
Zinc sulfate solution: Dissolve 100 g $ZnSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 liter.
Sodium hydroxide solution, 6N: Dissolve 240 g NaOH in 500 mL distilled water, cool and dilute to 1 liter.
Ammonium hydroxide, conc.
Dilute hydrochloric acid, 6N: Dilute 50 mL of conc. HCl to 100 mL with distilled water.
Copper sulfate solution, 2%: Dissolve 20 g of $CuSO_4 \cdot 5H_2O$ in 500 mL of distilled water and dilute to 1 liter.
Stock nitrate solution: Dissolve 7.218 g KNO_3 in distilled water and dilute to 1000 mL. Preserve with 2 mL of chloroform per liter. This solution is stable for at least 6 months. 1.0 mL = 1.00 mg NO_3^-N.
Standard nitrate solution: Dilute 10.0 mL of nitrate stock solution to 1000 mL with distilled water. 1.0 mL = 0.01 mg NO_3^-N.
Stock nitrite solution: Dissolve 6.072 g KNO_2 in 500 mL of distilled water and dilute to 1000 mL. Preserve with 2 mL of chloroform and keep under refrigeration. Stable for approximately 3 months. 1.0 mL = 1.00 mg NO_2^-N.
Standard nitrite solution: Dilute 10.0 mL of stock nitrite solution to 1000 mL with distilled water. 1.0 mL = 0.01 mg NO_2^-N. Using standard nitrate solution prepare the following standards in 100 mL volumetric flasks:

Conc. mg-NO_3^-N/L	mL of Stand Solution per 100.0 mL
0.00	**0.0**
0.05	**0.5**
0.10	**1.0**
0.20	**2.0**
0.50	**5.0**
1.00	**10.0**

Both nitrate and nitrite can be determined by this method. If only nitrate is desired, a separate determination must be made for nitrite, and nitrate is determined by difference. The nitrite may be determined by the procedure below without the reduction step. A filtered sample is passed through a reduction column containing granulated copper-cadmium, converting the nitrate to nitrite. The combined concentration of nitrite and nitrate is then determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo-dye which is spectrophotometrically measured. If the sample is not passed through the reduction column, only the nitrite will be measured. By analyzing the sample once and passing it through the column, the nitrate can be determined by difference.

6.8.1.1 Sample Preparation

Analysis should be performed as soon as possible. If the analysis cannot be made within 24 hours, the sample should be preserved with sulfuric acid (2 mL H_2SO_4 per liter) and refrigerated at 4ºC. However, once preserved, only nitrate + nitrite can be determined. Otherwise store refrigerated at 4ºC. Samples to be analyzed for nitrate with the reduction column must not be preserved with mercuric chloride. Interferences can come from several sources. Flow through the reduction column can be reduced due to build up of suspended matter. This is easily controlled by pre-filtering through a glass fiber filter or a 0.45 µm membrane filter. If samples are highly turbid samples, they may be pretreated with zinc sulfate before filtration to remove the bulk of particulate matter present in the sample. Samples containing high concentrations of iron, copper and other metals may be underestimated. A pre-treatment of EDTA can reduce the interference. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium and reduce the efficiency of the reduction step. Pre-extraction of the sample with an organic solvent will reduce the effects on the reduction step.

Removal of turbidity is accomplished by filtration of the sample or in severe cases through flocculation and subsequent filtering. Filter the sample through a glass fiber filter or a 0.45 µm membrane filter. For samples with high levels of suspended matter, add 1 mL zinc sulfate solution to 100 mL of sample and mix thoroughly. Add 0.4-0.5 mL sodium hydroxide solution to obtain a pH of 10.5 as determined with a pH meter. Let the treated sample stand a few minutes to allow the heavy flocculent to precipitate and settle. Clarify the sample by filtering it through a glass fiber filter or a 0.45 µm membrane filter.

Oil and grease removal is accomplished by a quick solvent extraction technique. Adjust the pH of 100 mL of filtered sample to 2 by addition of conc. HCl. Extract the oil and grease from the aqueous solution with two 25 mL portions of a non-polar solvent (i.e., chloroform or equivalent).

6.8.1.2 Sample Processing

The pH should be adjusted if the sample pH is below 5 or above 9 with either conc. HCl or conc. NH_4OH. This is done to insure a sample pH of 8.5 after the following step. To 25.0 mL of sample or an aliquot diluted to 25.0 mL, add 75 mL of ammonium chloride-EDTA solution and mix. Then,

- Pour the sample into the column and collect the sample at a rate of 7-10 mL per minute.
- Discard the first 25 mL, collect the rest of the sample (approximately 70 mL) in the original sample flask.
- Reduced samples should not be allowed to stand longer than 15 minutes before addition of color reagent.
- Add 2.0 mL of color reagent to 50.0 mL of sample.
- Allow 10 minutes for color development.
- Within 2 hours measure the absorbance at 540 nm against a reagent blank.

NOTE: If the concentration of sample exceeds 1.0 mg NO_3^-N/L, the remainder of the reduced sample may be used to make an appropriate dilution before proceeding with the next step. Carry out the reduction of standards exactly as described for the samples.

At least one nitrite standard should be compared to a reduced nitrate standard at the same concentration to verify the efficiency of the reduction column.

6.8.1.3 Calculations

Obtain a standard curve by plotting the absorbance of standards run by the above procedure against NO_3^-N mg/L. Compute concentration of samples by comparing sample absorbance with standard curve. If less than 25 mL of sample is used for the analysis, the following equation should be used:

$$NO_2 + NO_3 \text{ (mg N/L)} = (A \times 25)/\text{mL sample used}$$

where:

A = Concentration of nitrate from the standard curve.

6.8.2 Autoanalyzer Method

The following procedure is used to determine nitrite singly or nitrite and nitrate combined in fresh, saline, or wastewater. The applicable concentration range for this method is 0.05 to 10.0 mg/L nitrate-nitrite nitrogen. The concentration range may be extended by dilution of the sample. In this procedure, a filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is colorimetrically measured. Speciated, rather than combined nitrate-nitrite, values are readily obtained by carrying out the procedure first with, and then without, the Cu-Cd reduction step.

6.8.2.1 Sample Preparation

Analysis should be completed as soon as possible after collection. If analyses can be conducted within 24 hours, the sample should be preserved by refrigeration at 4°C.

"Apparatus, Materials, and Reagents - Nitrate/Nitrite"

- **Technicon or Equivalent AutoAnalyzer** (AAI or AAII) consisting of the following components:
  - Sampler
  - Manifold (AAI) or analytical cartridge (AAII).
  - Proportioning Pump
  - Colorimeter equipped with a 15 mm or 50 mm tubular flow cell and 540 nm filters.
  - Recorder
  - Digital printer.
- **Granulated cadmium**: 40-60 mesh (take extreme care in handling metallic cadmium, it is very toxic).

- **Copper-cadmium**. The cadmium granules (new or used) are cleaned with dilute HCl and copperized with 2% solution of copper sulfate in the following manner:
  - Wash the cadmium with HCl and rinse with distilled water. The color of the treated cadmium should be silver.
  - Swirl 10 g cadmium in 100 mL portions of 2% solution of copper sulfate for five minutes or until the blue color partially fades, decant and repeat with fresh copper sulfate until a brown colloidal precipitate forms.
  - Wash the cadmium-copper with distilled water (at least 10 times) to remove all of the precipitated copper. The color of the treated cadmium should be black.
- **Preparation of reduction column AAI**: The reduction column is an 8 by 50 mm glass tube with the ends reduced in diameter to permit insertion into the system. Copper-cadmium granules are placed in the column between glass wool plugs. The packed reduction column is placed in an up-flow 20° incline to minimize channeling (Figure 6.1).

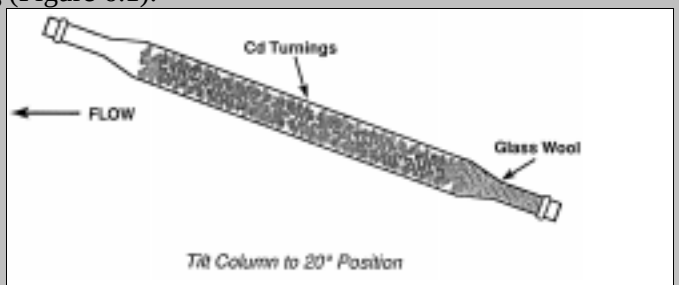

Figure 6.1. Copper Cadmium Reduction Column.

- **Preparation of reduction column**: The reduction column is a U-shaped, 35 cm length, 2 mm I.D. glass tube (Note 1). Fill the reduction column with distilled water to prevent entrapment of air bubbles during the filling operations. Transfer the copper-cadmium granules to the reduction column and place a glass wool plug in each end. To prevent entrapment of air bubbles in the reduction column be sure that all pump tubes are filled with reagents before putting the column into the analytical system.

  NOTE 1: A 0.081 I.D. pump tube (purple) can be used in place of the 2 mm glass tube.
- **Distilled water**. Because of possible contamination, distilled water should be prepared by passing it through an ion exchange column comprised of a mixture of both strongly acidic-cation and strongly basic-anion exchange resins. The regeneration of the ion exchange column should be carried out according to the manufacturer's instructions or Type I water by CAP/NCCLS.
- **Color reagent**. Add while stirring, 100 mL conc. phosphoric acid, 40 g sulfanilamide, and 2 g N-1-naphthylethylenediamine dihydrochloride to 800 mL of distilled water. Stir until all materials dissolve and then dilute to 1 liter. Store in a brown bottle and keep in the dark when not in use. This solution is stable for several months.
- **Dilute hydrochloric acid**, 6N: Dilute 50 mL of conc. HCl to 100 mL with distilled water.
- **Copper sulfate solution**, 2%: Dissolve 20 g of $CuSO_4 \cdot 5H_2O$ in 500 mL of distilled water and dilute to 1 liter.
- Wa**sh solution**. Use distilled water for unpreserved samples. For samples preserved with H_2SO_4, use 2 mL H_2SO_4 per liter of wash water.
- **Ammonium chloride-EDTA solution**. Dissolve 85 g of reagent grade ammonium

chloride and 0.1 g of disodium ethylenediamine tetraacetate in 900 mL of distilled water. Adjust the pH to 8.5 with conc. ammonium hydroxide and dilute to 1 liter. Add 1/2 mL Brij-35 (available from Technicon Corporation).

- **Stock nitrate solution**. Dissolve 7.218 g KNO_3 and dilute to 1 liter in a volumetric flask with distilled water. Preserve with 2 mL of chloroform per liter. This solution is stable for 6 months (1 mL = 1.0 mg NO_3^-N).
- **Stock nitrite solution**. Dissolve 6.072 g KNO_2 in 500 mL of distilled water and dilute to 1 liter in a volumetric flask. Preserve with 2 mL of chloroform and keep refrigerated (1.0 mL = 1.0 mg NO_2^-N).
- **Standard nitrate solution**. Dilute 10.0 mL of stock nitrate solution to 1000 mL. 1.0 mL = 0.01 mg NO_3^-N. Preserve with 2 mL of chloroform per liter. Solution is stable for 6 months.
- **Standard nitrite solution**. Dilute 10.0 mL of stock nitrite solution to 1000 mL. 1.0 mL = 0.01 mg NO_2^-N. Solution is unstable; prepare as required.
- Using the standard nitrate solution, prepare the following standards in 100.0 mL volumetric flasks. At least one nitrite standard should be compared to a nitrate standard at the same concentration to verify that the reduction column is efficiently operating.

Conc., mgNO,-N or NO_3-N/L	mL Standard Solution/100 mL
0.0	0
0.05	0.5
0.10	1.0
0.20	2.0
0.50	5.0
1.00	10.0
2.00	20.0
4.00	40.0
6.00	60.0

When the samples to be analyzed are saline waters, Substitute Ocean Water (SOW) should be used for preparing the standards; otherwise, distilled water is used. The composition of SOW is:

NaCl - 24.53 g/L	$MgCl_2$ - 5.20 g/L	Na_2SO_4 - 4.09 g/L
$CaCl_2$ - 1.16 g/L	KCl - 0.70 g/L	$NaHCO_3$ - 0.20 g/L
KBr - 0.10 g/L	H_3BO_3 - 0.03 g/L	$SrCl_2$ - 0.03 g/L
NaF - 0.003 g/L		

6.8.2.2 Sample Processing

If the pH of the sample is below 5 or above 9, adjust the pH to between 5 and 9 with either concentrated HCl or concentrated NH_4OH. Set up the manifold as shown in Figure 6.2 (AAI) or Figure 6.3 (AAII). Note that reductant column should be in 20º incline position (AAI). Care should be taken not to introduce air into the reduction column on the AAII. Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, flushing distilled water through the sample line. Condition the column by flushing 1 mg/L standard for 10 minutes when a new reduction column is first used. Subsequently wash the column with reagents for 20 minutes.

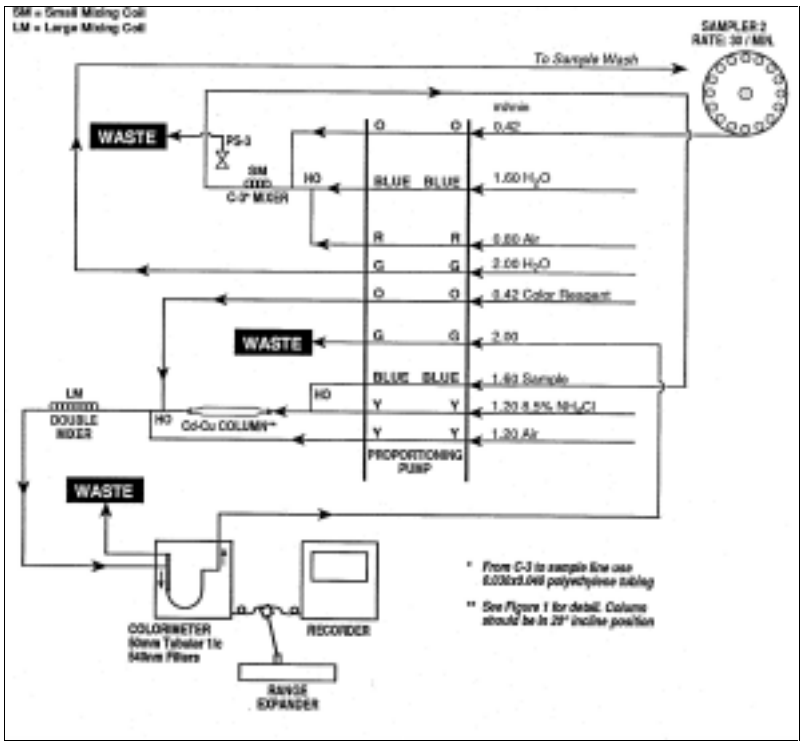

Figure 6.2. Nitrate-Nitrite Manifold AA-I.

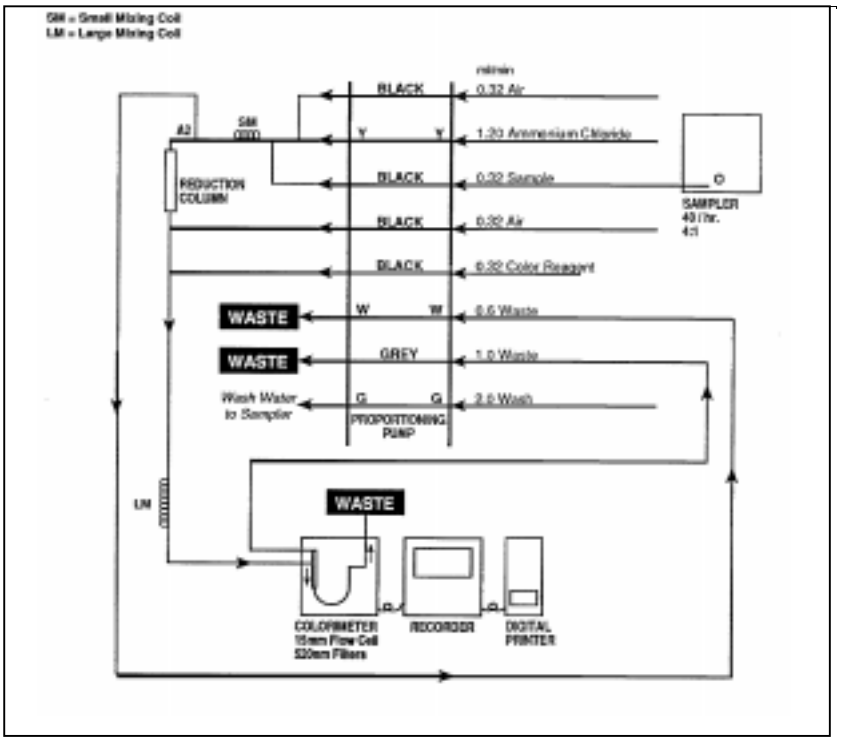

Figure 6.3. Nitrate-Nitrite Manifold AA-II.

Place the appropriate nitrate and/or nitrite standards in the sampler in order of increasing concentration of nitrogen. Complete loading of sampler tray with unknown samples and begin analyses.

6.8.2.3 Calculations

Prepare appropriate standard curve or curves derived from the NO_2^-N and/or NO_3^-N standards. Compute the concentration of samples by comparing sample peak heights with the standard curve.

The analyst should be aware that interferences with the method can be caused by a number of factors:

- The build-up of suspended matter in the reduction column will restrict sample flow. Since nitrate-nitrogen is dissolved, the sample may be pre-filtered.
- Low results may be obtained for samples that contain high concentrations of iron, copper or other metals. EDTA is added to the samples to eliminate this interference.
- Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

Autoanalyzer methods are also available for other nutrients. The analyst should consult the manufacturers' manual.

6.8.3 Portable Test Kits

There are portable test kits available for the nitrate/nitrite analysis that provide pre-packaged reagents that limit the necessity for large equipment purchases and limit the amount of chemical waste generated. This technique utilizes cadmium to reduce nitrates to nitrite in the sample, the nitrate reacts with sulfanilic acid in an acidic medium to form an intermediate diazonium salt. The absorbance at 500 nm is determined using either a calibrated bench or portable spectrophotometer. The working range for this method is 0 to 30.0 mg/L NO_3-N. The method is as follows:

"Apparatus, Materials, and Reagents - Nitrate/Nitrite"

- Portable or bench spectrophotometer with 500 nm filter (HACH 800 or similar Cat. No. 48470-00) or equivalent.
- Sample cells
- Bromine Water
- Nitrate Nitrogen Standard
- Phenol Solution
- Graduated cylinder
- Sodium Hydroxide

- Deionized Water
- Prepackaged Reagents
- Stopwatch

- Fill sample cell with 10 or 25 mL of sample (depending on instrument).
- Add pre-packaged reagent to the cell, stopper the cell.
- Shake for one minute.
- Add bromine water (30 g/L) dropwise to the sample until a yellow color remains.
- Add one drop of phenol solution (30 g/L), to remove color.
- Time reaction, 5 minutes.
- Fill another cell with 10 or 25 mL of sample (depending on instrument) as instrument blank.
- Zero instrument using instrument blank.
- Take sample reading.

The samples can be stored for up to 14 days at a pH equal to 2 (adjusted using sulfuric acid), in the dark, and refrigerated. Before analyzing stored samples, samples will need to be neutralized using sodium hydroxide. Strong oxidizing and reducing agents will interfere with the analysis. Ferric iron will bias the results high while chloride greater than 100 mg/L will bias the results low. The samples can also be analyzed using a color wheel to compare colors instead of a spectrophotometer; however; at least 20% more variability is introduced into the method. Pre-packaged portable laboratories are commercially available for a suite of water quality measurements that can be performed in the field. The analyses that can be performed with these type of pre-packaged field kits include acidity, alkalinity, aluminum, ammonia, carbon dioxide, chloride, chlorine dioxide, color, conductivity, copper, hardness, total iron, manganese, dissolved oxygen, pH, phosphate, silicate, sulfate, sulfide, suspended solids, temperature, turbidity and zinc.

6.8.4 Ion Selective Electrodes

Nitrate and nitrite concentrations can be measured by ion-selective electrode (ISE). The main advantage of electrode technology is the specificity and the speed that an analysis can be made. The electrode is immersed into the sample solution and the sample concentration is directly read on a specific ion meter or from a calibration curve. Time-consuming sample preparation steps such as filtration and chromium addition are unnecessary. Sample color or turbidity does not affect the measurements. Analysis time is typically under one minute. The ISE is connected to a either a benchtop or portable Ion Selective Meter and the meter is calibrated using calibration solutions. Nitrate and nitrite ions are measured using separate combination plastic membrane electrodes. The working range for a nitrate ISE is from 0.1 to 14,000 ppm and for a nitrite ISE is from 0.02 to 100 ppm. Samples should be analyzed as soon as possible, preferably in the field at the time of sampling.

"Apparatus, Materials, and Reagents - Ion Selective Electrodes"

- Ion Selective/pH Meter (similar to PCM700 ORION)
- Nitrate Combination ISE (ORION 9707BN)

- Nitrite Combination ISE (ORION 9746BN)
- Sample container
- Nitrate Standards
- Electrode filling Solution

There are numerous applications for ISE for the measurement of a large suite of analytes. Ion selective electrodes are used to serve as endpoint indicators for titrations for analytes including: aluminum, arsenic, azide, boron, chloride, chromium, iron, lithium, manganese, nickel, nitroloacetate, sulfate, and sulfur. The results using ISE as an indicator for analyses involving titrations are approximately ten times more precise than direct measurements, but more time consuming. Direct measurements made with ion selective electrodes, where the probe is immersed in the sample, is a very convenient field method. The meter is typically calibrated by measurement of a series of standards and the sample reading taken so that it is within the standard curve. Samples with readings outside the standard curve would require an appropriate dilution to put the response within the calibration curve. Analytes suitable for direct measurement include: ammonia, calcium, carbon dioxide, chloride, copper (with std addition), cyanide, fluoride, potassium, silver, sodium, and sulfide. Each ISE will have specific storage requirements (e.g. soaking in low level standard, buffer, etc) for between samples, short-term storage (up to one week), and long-term storage. The manufacturer can provide information on range of operation, storage requirements, and method documentation.

6.8.5 Test Strips

Water quality test strips are commercially available (HACH Cat. No. 27454-25, ORION Cat No. T2080P or equivalent) for performing semi-quantitative nitrate/nitrite determinations. The test strips provide two reagent pads (for both nitrate and nitrite) on a single test strip. These test strips give fast and reliable results that are very cost effective and eliminate the need for clean up or disposal of waste chemicals often associated with the classical wet chemical methods described above. The procedure for using these strips follows:

- The test strip is dipped into the sample.
- Wait for the color to develop
- Compare the color of the reagent pads to the color chart provided with the test strips for the nitrate and nitrite concentration.

The range for NO_3-N is 0 to 50 mg/L (with increments of 0, 1, 2, 5, 10, 20, and 50 mg/L for HACH) or 0 to 500 mg/L (with increments of 0, 10, 25, 50, 100, 250, 500 mg/L for ORION). For NO_2-N the range is 0 to 3 ppm (with increments of 0, 0.15, 0.3, 1.0, 1.5, and 3.0 for HACH) and 0 to 80 (with increments of 0, 1, 5, 10, 25, 40, and 80 mg/L for ORION). This technique is best suited to distinguish between large differences in nitrate/nitrite concentrations that would be typically encountered when determining the spatial distribution around a sewage outfall and not defining fine scale variability. Test strips are also commercially available for the determination of total alkalinity (0-240 mg/L), free and total chlorine (0-10 mg/L), total hardness (0-425 mg/L), total iron (0-5 mg/L), total copper (0-3 mg/L), ammonia (0-6 mg/L), and phosphate (0-50 mg/L).

6.9 Phosphate

Phosphorous is an additional essential micronutrient for biological organisms. As with nitrogen, the introduction of phosphorous into waters where it is growth-limiting can cause a proliferation of biological production contributing to eutrophication. Dissolved phosphorous is primarily in the form of phosphate.

"Apparatus, Materials, and Reagents - Phosphate"

- **Photometer**. A spectrophotometer or filter photometer suitable for measurements at 650 or 880 nm with a light path of 1 cm or longer.
- **Acid-washed glassware**. All glassware used should be washed with 1:1 HCl and rinsed with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove the last traces of phosphorus that might be adsorbed on the glassware. Preferably, this glassware should be used only for the determination of phosphorus and after use it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with 1:1 HCl and reagents is only required occasionally. Commercial detergents should never be used.
- **Sulfuric acid solution**, 5N: Dilute 70 mL of concentrated H_2SO_4 with distilled water to 500 mL.
- **Antimony potassium tartar solution**. Weigh 1.3715 g $K(SbO)C_4H_4O_6 \bullet 1/2H_2O$ dissolve in 400 mL distilled water in 500 mL volumetric flask, dilute to volume. Store at 4ºC in a dark, glass-stoppered bottle.
- **Ammonium molybdate solution**. Dissolve 20 g $(NH_4)_6Mo_7O_{24} \bullet 4H_2O$ in 500 mL of distilled water. Store in a plastic bottle at 4ºC.
- **Ascorbic acid**, 0.1 M: Dissolve 1.76 g of ascorbic acid in 100 mL of distilled water. The solution is stable for about a week if stored at 4ºC.
- **Combined reagent**. Mix the above reagents in the following proportions for 100 mL of the mixed reagent: 50 mL of 5N H_2SO_4, 5 mL of antimony potassium tartrate solution, 15 mL of ammonium molybdate solution and 30 mL of ascorbic acid solution. Mix after addition of each reagent. All reagents must reach room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until the turbidity disappears before proceeding. Since the stability of this solution is limited, it must be freshly prepared for each run.
- **Sulfuric acid solution**, 11 N: Slowly add 310 mL conc. H_2SO_4 to 600 mL distilled water. When cool, dilute to 1 liter.
- **Stock phosphorus solution**. Dissolve in distilled water 0.2197 g of potassium dihydrogen phosphate, KH_2PO_4, which has been dried in an oven at 105ºC. Dilute the solution to 1000 mL; 1.0 mL = 0.05 mg PO_4-P.
- **Standard phosphorus solution**. Dilute 10.0 mL of stock phosphorus solution to 1000 mL with distilled water; 1.0 mL=0.5 µg PO_4-P. Using standard solution, prepare the following standards in 50.0 mL volumetric flasks:

mL of Standard Phosphorus Solution	Conc., mg/L
0	0.00
1.0	0.01
3.0	0.03

5.0	0.05
10.0	0.10
20.0	0.20
30.0	0.30
40.0	0.40
50.0	0.50

- **Sodium hydroxide,** 1 N: Dissolve 40 g NaOH in 600 mL distilled water. Cool and dilute to 1 liter.

The reaction that forms the basis for this analysis is that of orthophosphate with antimony and molybdate to produce an antimony-phospho-molybdate complex. Depending on the pretreatment of the sample, the various forms of phosphorus may be determined. The most commonly measured forms are phosphorus and dissolved phosphorus and orthophosphate and dissolved orthophosphate. Hydrolyzable phosphorus is normally found only in sewage-type samples and the insoluble forms of phosphorus are determined by calculation. The concentration range for this method is between 0.01 to 1.2 mg PO_4-P.

This method can be used for the determination of phosphorus in drinking, surface and saline waters, domestic and industrial wastes and is adapted from EPA Method 365.2.

6.9.1 Sample Preparation

Samples can be collected in either clean plastic or glass containers. If analysis can not be performed within 24 hours, the sample should be preserved with 2.0 mL of concentrated H_2SO_4 per liter of sample and refrigerated at 4ºC. Various forms of phosphorus can be determined using different pretreatments.

For total phosphorus determinations:

- Add 1 mL of H_2SO_4 solution to a 50 mL sample in a 125 mL Erlenmeyer flask.
- Add 0.4 g of ammonium persulfate.
- Boil gently on a pre-heated hot plate for approximately 30 minutes or until a final volume of about 10 mL is reached.
- Do not allow sample to go to dryness.

Alternatively:

- Heat for 30 minutes in an autoclave at 121ºC (15-20 psi).
- Cool and dilute the sample to about 30 mL and adjust the pH of the sample to 7.0 ± 0.2 with 1 N NaOH (7.10) using a pH meter.
- If the sample is not clear at this point, add 2-3 drops of acid and filter. Dilute to 50 mL.

For hydrolyzable phosphorus determinations:

- Add 1 mL of H_2SO_4 solution to a 50 mL sample in a 125 mL Erlenmeyer flask.
- Boil gently on a pre-heated hot plate for 30 minutes or until a final volume of about 10 mL is reached. Do not allow sample to go to dryness.

Alternatively:

- Heat for 30 minutes in an autoclave at 121ºC (15-20 psi).
- Cool and dilute the sample to about 30 mL and adjust the pH of the sample to 7.0 ± 0.2 with NaOH using a pH meter.
- If sample is not clear at this point, add 2-3 drops of acid and filter.
- Dilute to 50 mL. The sample is now ready for determination of phosphorus.

6.9.2 Sample Processing

For orthophosphate determinations:

- Adjust to a pH of 7 ± 0.2 using a pH meter.
- Add 8.0 mL of combined reagent to the sample and thoroughly mix.
- After a minimum of ten minutes, but no longer than thirty minutes, measure the color absorbance of each sample at 650 or 880 nm with a spectrophotometer, using the reagent blank as the reference solution.

NOTE: If the same volume of sodium hydroxide solution is not used to adjust the pH of the standards and samples, a volume correction must be made.

6.9.3 Calculations

Prepare a standard curve by plotting the absorbance values of standards versus the corresponding phosphorus concentrations. Process standards and blank exactly as the samples. Analyze at least one blank and two standards with each series of samples. If the standards do not agree within ± 2% of the known value, prepare a new calibration curve. Obtain the concentration value of the sample directly from the standard curve. Report results as PO_4-P, mg/L.

6.10 Temperature (T)

In addition to having its own biological effects (i.e., thermal pollution), temperature affects the solubility and, in turn, the toxicity of many other constituents. For example, the solubility of solids increases with increasing temperature, while gases tend to be more soluble in cold water. Thermal pollution is often cited as a cause of environmental degradation. Many thermal pollution problems are a result of anthropogenic activities; however, some water quality problems can occur due to natural fluctuations in temperature. This method is applicable to marine waters, freshwaters, and wastewaters.

The following method is adapted from: US EPA. 1998. Understanding Environmental Methods: Analytical Methods, Environmental Analysis, and Data Evaluation: EPA Method

170.1 Approved for NPDES (Issued 1974); TITLE: Temperature (Thermometric). © Genium Publishing Corporation 1996, Schenectady, NY 12304.

Temperature measurements may be made with any good grade of mercury-filled or dial-type centigrade thermometer, or a thermistor. Measurement device should be routinely checked against a certified precision thermometer.

PERFORMANCE

Precision and accuracy for this method have not been determined. The procedure to be used for this determination is found in: Standard Methods for the Examination of Water and Wastewater, 14th Edition, p. 125, Method 212 (1975).

6.11 Coliform Bacteria

The membrane filter method can be applied for the determination and enumeration of the coliform bacteria in freshwater, marine waters, and wastewaters. A membrane filter is placed on the specified mEI agar medium and incubated at 41ºC for 24 h. Using magnification and a fluorescent lamp, coliform are identified as those bacteria that produce colonies with a blue halo after incubation on mEI agar. The presence of coliform in water is an indication of fecal pollution and the presence of enteric pathogens, and shows a direct relationship between the coliform density in water and waste discharges. Measurement of living bacteria requires substantial infrastructure support and may not be able to be performed at locations with little infrastructure.

6.11.1 Sample Preparation and Processing

Sample preservation procedures and holding time limits must be carefully followed to produce valid data. Bacteriological samples must be refrigerated at a temperature of 1-4ºC during transit to the laboratory. Insulated containers assure proper maintenance of storage temperature. Sample bottles should not be immersed in water during transit or storage.

Examine samples as soon as possible after collection. Do not hold samples longer than 6 hours between collection and the initiation of the analyses.

"Apparatus, Materials, and Reagents - Coliform Bacteria"

- **Glass lens** with magnification of 2-5X or stereoscopic microscope.
- **Lamp**, with a cool, white fluorescent tube.
- **Hand tally or electronic counting device**.
- **Pipet container**, stainless steel, aluminum or borosilicate glass, for glass pipets.
- **Pipets**, sterile, bacteriological or Mohr, glass or plastic, of appropriate volume.
- **Graduated cylinders**, 100-1000 mL, covered with aluminum foil or kraft paper and sterile.
- **Membrane filtration units** (filter base and funnel), glass, plastic or stainless steel, wrapped with aluminum foil or kraft paper and sterile.
- **Ultraviolet unit for sanitization of the filter funnel** between filtrations (optional).

- **Line vacuum, electric vacuum pump, or aspirator** for use as a vacuum source. In an emergency or in the field, a hand pump or a syringe equipped with a check valve to prevent the return flow of air, can be used.
- **Flask, filter, vacuum**, usually 1 L, with appropriate tubing. A filter manifold to hold a number of filter bases is optional.
- **Flask for safety trap** placed between the filter flask and the vacuum source.
- **Forceps**, straight or curved, with smooth tips to handle filters without damage.
- **Ethanol, methanol or isopropanol** in a small, wide-mouth container, for flame-sterilizing forceps.
- **Burner, Bunsen or Fisher type**, or electric incinerator unit for sterilizing loops and needles.
- **Thermometer**, checked against a National Institute of Standards and Technology (NIST) certified thermometer, or one that meets the requirements of NIST Monograph SP 250-23.
- **Petri dishes**, sterile, plastic, 50 x 12 mm, with tight-fitting lids.
- **Bottles, milk dilution, borosilicate glass, screw-cap with neoprene liners**, marked at 99 mL for 1-100 dilutions. Dilution bottles marked at 90 mL or tubes marked at 9 mL may be used for 1-10 dilutions.
- **Flasks, borosilicate glass**, screw-cap, 250-2000 mL volume.
- **Membrane filters**, sterile, white, grid marked, 47 mm diameter, with 0.45 + 0.02 µm pore size.
- **Inoculation loops**, at least 3-mm diameter, and needles, nichrome or platinum wire, 26 B & S gauge, in suitable holders.
- **Incubator** maintained at 41 ± 0.5ºC.
- **Waterbath** maintained at 44-46ºC for tempering agar.
- **Test tubes**, 150 x 20 mm, borosilicate glass or plastic.
- **Caps, aluminum or autoclavable plastic**, for 20 mm diameter test tubes.
- **Test tubes, screw-cap, borosilicate glass**, 125 x 16 mm or other appropriate size.
- **Purity of Reagents**. Reagent grade chemicals shall be used in all tests. The agar used in preparation of culture media must be of microbiological grade. Whenever possible, use commercial culture media as a means of quality control.
- **Purity of Water**. Reagent water conforming to Specification D1193, Reagent water conforming Type II, Annual Book of ASTM Standards.

- **Buffered Dilution Water Composition**

Sodium Dihydrogen Phosphate	0.58 g
Sodium Monohydrogen Phosphate	2.50 g
Sodium Chloride	8.50 g

Dissolve the ingredients in 1 L of reagent water in a flask and dispense in appropriate amounts for dilutions in screw-cap bottles or culture tubes and/or into containers for use as rinse water. Autoclave after preparation at 121ºC for 15 min. The final pH should be 7.4 ± 0.2.

- **mEI Agar Composition of Basal Medium (mE Agar, Difco 0333):**

Peptone	10.0 g
Sodium Chloride	15.0 g
Yeast Extract	30.0 g
Esculin	1.0 g
Actidione	0.05 g

Sodium Azide	0.15 g
Agar	15.0 g

Add 71.2 g of dehydrated basal medium plus 0.75 grams of indoxyl B-D glucoside to 1 L of reagent grade water in a flask and heat to boiling until ingredients dissolve. Autoclave at 121ºC for 15 min and cool in a 44-46ºC water bath. Mix 0.24 g nalidixic acid in 5 mL reagent grade water, add a few drops of 0.1N NaOH to dissolve; add to the mEI medium. Add 0.02 g triphenyl tetrazolium chloride separately to the mEI medium and mix. Pour the mEI agar into 50 mm petri dishes to a 4-5 mm depth (approximately 4-6 mL), and allow to solidify. The final pH of medium should be 7.1 ± 0.2. Store in a refrigerator.

- **Brain Heart Infusion (BHI) (Difco 0037-02, BBL 11058) Composition**

Calf Brain Infusion	200.0 g
Beef Heart Infusion	250.0 g
Peptone	10.0 g
Sodium Chloride	5.0 g
Disodium Phosphate	2.5 g
Dextrose	2.0 g

Dissolve 37 g of dehydrated brain heart infusion in 1 L of reagent grade water. Dispense in 8-10 mL volumes in screw-cap tubes and autoclave at 121ºC for 15 min. If the medium is not used the same day as prepared and sterilized, heat in boiling water bath for several minutes to remove absorbed oxygen, and cool quickly without agitation, just prior to inoculation. The final pH should be 7.4 ± 0.2.

- **Brain Heart Infusion (BHI) Broth with 6.5% NaC1**

Brain heart infusion broth with 6.5% NaC1 is the same as BHI broth in 7.6 with additional NaC1. Add 60.0 g NaC1 per liter of medium. Since most commercially available dehydrated media contain sodium chloride, this amount is taken into consideration in determining the final NaCl percentage above.

- **Brain Heart Infusion Agar (Difco 0418-02, BBL 11064)**

Brain heart infusion agar contains the same components as BHI (see 7.6) with the addition of 15.0 g of agar per L of BHI broth. Heat to boiling until ingredients are dissolved. Dispense 10-12 mL of medium in screw-cap test tubes and sterilize for 15 min at 121ºC. Slant after sterilization. The final pH should be 7.4 ± 0.2.

- **Bile Esculin Agar (BEA) (Difco 0879) Composition:**

Bacto Beef Extract	3.0 g
Bacto Peptone	5.0 g
Bacto Oxgall	40.0 g
Bacto Esculin	1.0 g
Ferric Citrate	0.5 g
Bacto Agar	15.0 g

Add 64.5 g of dehydrated BEA to 1 L reagent water and heat to boiling to dissolve completely. Dispense in 8-10 mL volumes in tubes for slants or into flasks for subsequent plating. Autoclave at 121ºC for 15 min. Overheating may cause

darkening of the medium. Cool to 44-46ºC and dispense into sterile petri dishes. The final pH should be 6.6 ± 0.2. Store in a refrigerator.

Care must be taken because suspended particulates can clog the membrane filters preventing filtration and contaminating the sample with non-target bacterial colonies. Mouth-pipetting is prohibited. Check temperatures in the incubators daily to ensure that operation is within the stated limits. Check thermometers at least annually against certified thermometers. Check mercury columns for breaks. Prepare the mEI agar as directed.

To prepare for analyses:

- Mark the petri dishes and report forms with sample identification and sample volumes.
- Place a sterile membrane filter on the filter base, grid-side up and attach the funnel to the base. The membrane filter is now held between the funnel and the base.
- Shake the sample bottle vigorously about 25 times to distribute the bacteria uniformly, and measure the desired volume of sample or dilution into the funnel.

For ambient surface waters and wastewaters, select sample volumes based on previous knowledge to produce 20-60 coliform colonies on the membranes. Sample volumes of 1-100 mL are normally tested at half log intervals, for example 100, 30, 10, 3 mL, etc. Smaller sample size or sample dilution can be used to minimize the interference of turbidity or high bacterial densities. Multiple volumes of the same sample or dilution of sample may be filtered and the results combined.

- Filter the sample and rinse the sides of the funnel at least twice with 20-30 mL of sterile buffered rinse water.
- Turn off the vacuum and remove the funnel from the filter base.
- Use sterile forceps to aseptically remove the membrane filter from the filter base and roll it onto the mEI agar to avoid the formation of bubbles between the membrane and the agar surface.
- Reseat the membrane if bubbles occur.
- Close the dish, invert, and incubate at 41 ± 0.5ºC for 24 h.
- After incubation, count and record colonies on those membrane filters containing, if practical, 20-60 colonies with any blue halo regardless of colony color as a coliform colony.

6.11.2 Calculations

Colonies with any blue halo can be verified as coliform. Use magnification for counting and a small fluorescent lamp to give maximum visibility of the colonies. Report the results as coliform per 100 mL of sample. Verification of colonies may be required in evidence gathering, and is also recommended as a QC procedure upon initial use of the test and with changes in sample sites or lots of commercial media. Using a sterile inoculating needle, transfer cells from the centers of at least 10 well-isolated typical colonies into a brain heart infusion broth (BHI) tube and onto a BHI slant. Incubate broth tubes for 24 h and slants for 48 h at 35 ± 0.5ºC. After 24 h incubation, transfer a loopful of material from each BHI broth tube to:

Bile Esculin Agar (BEA) and incubate at 35± 0.5ºC for 48 h.
BHI Broth and incubate at 45 ± 0.5ºC for 48 h.
BHI Broth with 6.5% NaC1 and incubate at 35 ± 0.5ºC for 48 h.

Observe for growth. After 48 h incubation, apply a gram stain to growth from each BHI agar slant. Gram positive cocci which grow in BEA, BHI Broth at 45ºC, and BHI Broth + 6.5% NaCl, and hydrolyze esculin, are verified as coliform.

PERFORMANCE

The specificity of the medium used in this method is 6.0% false positive and 6.5% false negative for various environmental water samples. The false positive rate was calculated as the percent of colonies which reacted typically, but did not verify as members of the coliform group. The false negative rate was calculated as the percent of all verified coliform colonies not reacting typically. The persistent positive or negative deviation of the results from the assumed or accepted true value is not significant. The precision among laboratories for marine water and surface water was 2.2% and 18.9%.

6.12 Phytoplankton

This method is based on the use of the Turner fluorometer or equivalent as suggested by Yentsch and Menzel (1963) and formalized by Holm-Hansen et al. (1965). This method determines chlorophyll *a* and is not as accurate as the spectrophotometric method. However, it is convenient and requires much smaller sample volumes for a given sensitivity than the spectrophotometric method. This method is adapted from that in Strickland and Parsons (1972).

"Apparatus, Materials, and Reagents - Phytoplankton"

- **The Turner fluorometers or equivalent:** fitted with the "high sensitivity" door equipped with F.4T4-BL lamp, Wratten 47B or Corning CS.5-60 filter for the excitation light and Corning CS.2-64 filter for the emitted light.
- **Millipore or equivalent filtration equipment:** designed to hold 47-mm diameter glass fiber filters.
- **47-mm Whatman or equivalent GF/C glass fiber filters.**
- **One 300-mL polyethylene wash bottle**.
- **Stoppered graduated centrifuge tubes**: 15-mL capacity having both glass and polyethylene stoppers.
- **Reagent grade acetone (90%):** 100 mL of water is added to a liter volumetric flask and acetone added to make the volume to exactly 1000 mL.
- **Magnesium carbonate suspension**. Add approximately 1 g of finely powdered magnesium carbonate ("Levis" grade) of analytical reagent quality to 100 mL of distilled water in a polyethylene bottle. Shake vigorously to resuspend the carbonate before each use.
- **5% v/v hydrochloric acid**

6.12.1 Sample Preparation

Samples should be obtained from the euphotic zone for phytoplankton. The final sample (500 mL to 5 liters) is filtered through a small piece of clean 0.3-mm mesh nylon netting to

remove the larger zooplankton. For open ocean samples, filtration of small volumes through a 0.15-mm mesh net will still not retain significant amounts of phytoplankton. The volume of filtrate should be measured and placed in a polyethylene bottle. A small amount of magnesium carbonate suspension (1.0 mL/liter) is added. The sample may then be stored in a refrigerated, dark place for a maximum of about 8 hr if the sample cannot be immediately filtered.

A blank is not necessary in this method since the instrument is specific for chlorophyll and this is not a contaminant found in reagents. The only background signal comes from scatter from the solvent (90% acetone) and it is negligible. The instrument is zeroed against a tube of 90% acetone with all doors closed immediately prior to use. Blanks should also be routinely analyzed to check instrument drift.

6.12.2 Sample Processing and Calculations

The water sample is processed as follows:

- Filter the sample by inverting the polyethylene bottle containing the sample into the funnel of the Millipore filter equipment fitted with a 47 mm Whatman GF/C glass fiber filter paper or equivalent.
- If not added previously, introduce about 1 mL of magnesium carbonate suspension to the last few hundred milliliters of sample being filtered.
- Drain the filter thoroughly under suction before removing it from the filtration equipment.
- Filters can be stored by folding them in half (with the plankton innermost), wrapping in aluminum foil and storing them in the dark at -20ºC but only for a few weeks.
- Store the filter if necessary but if possible extract the pigment without delay.
- Place the filter in a 15-mL stoppered graduated centrifuge tube.
- Add approximately 10 mL of 90% acetone, stopper the tube, and disperse and disaggregate the filter by shaking the tube vigorously.
- Allow the pigments to be extracted by placing the tube in a refrigerator in complete darkness for about 20 hr.
- Shake the tubes vigorously once more after they have been 1 or 2 hr in the refrigerator.
- Remove tubes from the refrigerator and let them warm up in the dark to room temperature. Add 90% acetone to make the extracts exactly 12.0 mL.
- Centrifuge the content of the tubes for 5-10 minutes having replaced the glass stoppers on the centrifuge tubes with plastic stoppers to prevent breakage during centrifugation.

Calibration must be done on each instrument. This is done with commercially pure chlorophyll *a*. This method is almost exclusively sensitive to chlorophyll *a* but there is a slight and variable response to other chlorophyll compounds. Dissolve a known amount of chlorophyll *a* in 90% acetone. This solution is then diluted quantitatively to yield a reading of about 50 on door 3 of the fluorometer (R_3). From this the correction factor (F_3) is determined:

$$F_3 = C_a/R_3$$

where C_a is the final concentration of chlorophyll *a* in the solution. Again, quantitatively dilute the standard solution in 90% acetone in order to get readings greater than 50 for doors 10 and 30. Calculate F_{10} and F_{30} from similar equations as above.

The phaeo-pigment ratio, τ, is the ratio of the readings R_B/R_A, obtained on the extract before and after acidification of the extract with 2 drops of 5% v/v hydrochloric acid. The standard solution should be diluted to get a reading of about 100 for R_B. The tube is acidified and the tube inverted several times and the reading R_A is obtained when the reading is stable. The ratio τ is then calculated:

$$\tau = R_B/R_A$$

This ratio should be near to 2.2 with the equipment specified and is obtained by making all measurements on one door. The ratio will not be constant for all samples if significant chlorophyll *c* is present, so slightly negative values for phaeo-pigments may sometimes be obtained. A single formula is sufficient, however, for most field work and will estimate the fraction of phaeo-pigments present in samples sufficiently well for most ecological studies.

Sample extracts are measured by pouring the clear supernatant into the cell and taking the readings with the appropriate door. The fluorometer should be checked for zero on a regular basis. This should be done prior to analysis and after every 10 samples and at the end of all readings. Provided that phaeo-pigments are absent:

$$\text{mg chlorophyll } a/m^3 = F_D \times R$$

If phaeo-pigments are present the concentration is calculated with both readings, before and after acidification. After the initial reading (R_B) of the fluorometer, 2 drops of 5% v/v hydrochloric acid are added and the cell is inverted several times to mix the solution. A second reading (R_A)is made. The sample should be left in the fluorometer long enough for the reading to become stable. This will insure complete conversion of all the chlorophyll to phaeophytin. The concentration of chlorophyll *a* and phaeophytin a are then determined by the equations:

$$\text{mg chlorophyll } a/m^3 = F_D \times (\tau / (\tau - 1)) \times (R_B - R_A) \times (V_e/V_f)$$

and

$$\text{mg phaeo-phytin a}/m^3 = F_D \times (\tau / (\tau - 1)) \times ((\tau \times R_A) - R_B) \times (V_e/V_f)$$

V_e = volume of extract
V_f = volume filtered

where R_B is the reading of the fluorometer before acidification, R_A is the reading after acidification at the same door, F_D is a factor for each door and τ is the factor determined during calibration.

Do not use solutions which necessitate the use of door 1 or which give readings much

greater than 50 on door 3. With such solutions the concentrations of chlorophyll are out of linearity between fluorescence and concentration. If solutions are too concentrated, dilute 3 mL (pipette) of extract with 3 mL of 90% acetone in a second clean, dry tube. Rinse tubes several times with water and 90% acetone to ensure removal of all acidic residues that could destroy the chlorophyll.

6.12.3 Modified Method for Chlorophylls and Carotenoids

This method describes the determination of the three chlorophylls commonly found in planktonic algae: chlorophylls *a*, *b*, and *c*. The carotenoid pigments (the carotenes and xanthophylls) can only be estimated in arbitrary units. If the phytoplankton population contains many myxophyceae some forms of phycobilin pigments may also extract and interfere with all determinations except that of chlorophyll *a*. This is a rare occurrence in marine environments.

This technique follows the method described by Richards (1952) with later modifications from Creitz (1955) and is described in Strickland and Parsons (1972). The original "specific plant unit" (SPU) defined by Richards approximates 1 g of dry pigment. The Richards equations, however, are capable of improvement in the light of more recent research. The original equations included are also from Parsons and Strickland (1963) and the SCOR/UNESCO (1966) Working Group on photosynthetic pigments.

This method is adequate where the chlorophyll content of the water is above 0.2 mg/m^3. Precision decreases rapidly below this level. These levels can be changed by filtering more or less seawater.

Finally mention should be made of chlorophyll degradation products. The presence of chlorophyllide will go undetected and this pigment will be reported as an equivalent weight of chlorophyll. If phaeophytin or phaeophorbide are present in samples the extinction of 665 nm will decrease and these pigments will go undetected and will be reported as if about half the amount of chlorophyll were present. Some idea of the amounts of phaeo-pigments present in a sample may be obtained by measuring extinctions before and after the acidification of extracts. Chlorophyll degradation products in samples of seawater are best determined by chromatography but this approach is lengthy and not suitable for routine application. The presence of such compounds can generally be ignored but large amounts may be found if bottom deposits are disturbed, if there has been very heavy grazing by zooplankton, or if samples are taken from just below the euphotic zone in the open sea.

6.12.3.1 Sample Preparation

The larger zooplankton are removed by straining a sample of seawater through a nylon net of about 300-µ mesh size and then the phytoplankton are filtered onto a Millipore AA filter or a glass fiber filter or equivalent. Pigments are extracted from the algae cells for spectrophotometric estimation.

"Apparatus, Materials, and Reagents - Chlorophylls"
• **Millipore filtration equipment**: designed to hold 47-mm diam membrane filters. • **One 300-mL polyethylene wash bottle.**

- **Stoppered graduated centrifuge tubes**: 15-mL capacity having both glass and polyethylene stoppers.
- "**Small volume" spectrophotometer cells** with a path length of 10 cm but holding 10 mL or less of solution.
- **Reagent grade acetone (90%)**: 100 mL of water is added to a liter volumetric flask and acetone added to make the volume to exactly 1000 mL.
- **Magnesium carbonate suspension**. Add approximately 1 g of finely powdered magnesium carbonate ("Levis" grade) of analytical reagent quality to 100 mL of distilled water in a polyethylene bottle. Shake vigorously to resuspend the carbonate before each use.
- **Dilute hydrochloric acid**. Dilute 50 mL of concentrated hydrochloric acid to 100 mL with distilled water.
- **47-mm diam Millipore AA filter**
- **A spectrophotometer**: capable of handling "small volume" spectrophotometer cells having a path length of 10 cm but holding 10 mL or less of solution.

6.12.3.2 Sample Processing

Samples should be obtained from the euphotic zone for phytoplankton. The final sample (500 mL to 5 liters) is filtered through a small piece of clean 0.3-mm mesh nylon netting to remove the larger zooplankton. For open ocean samples filtration of small volumes through a 0.15-mm mesh net will still not retain significant amounts of phytoplankton. The volume of filtrate should be measured and placed in a polyethylene bottle. A small amount of magnesium carbonate suspension (1.0 mL/liter) is added. The sample may then be stored in a refrigerated dark place for a maximum of about 8 hr if the sample can not be filtered immediately. Once the sample is filtered, the membrane filters can be stored by folding them in half (with the plankton innermost) and storing them in the dark in a desiccator frozen to -20^{o}C but only for a few weeks. This procedure almost always leads to low results and makes the extraction of chlorophyll more difficult; filters should be extracted without delay if at all possible.

After processing as described above, the clear supernatant of the sample extracts are then poured into the 10-cm-path-length cell and readings are taken with the appropriate door. When extinction values exceed 1.3, the measurements described below should be made with 2.5-cm or 1-cm cells and the extinction values multiplied by 4 or 10, respectively, to convert them to the values expected with a 10-cm cell. Without delay measure the extinction of the solution against a cell containing 90% acetone at 750, 665, 645, 630, and 480 nm. If the Richards equations are to be used for carotenoids (see below) a further measurement at 510 nm is required, and if the SCOR/UNESCO equations are used the measurement at 665 nm should be replaced by one at 663 nm. Record the extinction values to the nearest 0.001 unit in the range 0-0.4 and the nearest 0.005 for extinction values exceeding 0.4. Correct the extinctions at each wavelength by the procedure described for calculating the turbidity blank for the millipore filter material.

6.12.3.3 Calculations

The concentration of pigments in water can then be calculated as:

$$\text{mg (or m-SPU) pigment/m}^3 = C/V$$

where C is a value obtained from the following equations and V is the volume of seawater

filtered in liters. When the Parsons-Strickland equations are used values for chlorophylls *a*, *b*, or *c* and carotenoids will be in mg/m^3 (R=Richards, P.S.=Parsons and Strickland, S.U.=SCOR/UNESCO). If the "classical" Richards equations are used values are in mg/m^3 for chlorophylls a and b. The Richards m-SPU is used for chlorophyll *c* and is considerably greater than the milligram. The m-SPU is considerably smaller than the milligram if carotenoids are mainly fucoxanthin or peridinin which are present in Chrysophyta or Pyrrophyta. In the formulae *E* stands for the extinction values, at wavelengths indicated by the subscripts, measured in 10-cm cells after correcting for a blank as described below.

R. C (chlorophyll *a*) = $15.6\ E_{665} - 2.0\ E_{645} - 0.8\ E_{630}$
P.S. C (chlorophyll *a*) = $11.6\ E_{665} - 1.31\ E_{645} - 0.14\ E_{630}$
S.U. C (chlorophyll *a*) = $11.64\ E_{663} - 2.16\ E_{645} + 0.10\ E_{630}$

R. C (chlorophyll *b*) = $25.4\ E_{645} - 4.4\ E_{665} - 10.3\ E_{630}$
P.S. C (chlorophyll *b*) = $20.7\ E_{645} - 4.34\ E_{665} - 4.42\ E_{630}$
S.U. C (chlorophyll *b*) = $20.97\ E_{645} - 3.94\ E_{663} - 3.66\ E_{630}$

R. C (chlorophyll *c*) = $109\ E_{630} - 125\ E_{665} - 287\ E_{645}$
P.S. C (chlorophyll *c*) = $55\ E_{630} - 4.64\ E_{665} - 16.3\ E_{645}$
S.U. C (chlorophyll *c*) = $54.22\ E_{630} - 14.81\ E_{645} - 5.53\ E_{663}$

R. C (Plant carotenoids) = $7.6\ (E_{480} - 1.49\ E_{510})$, without regard to species
P.S. C (Plant carotenoids) = $4.0\ E_{480}$ (if species are predominately Chlorophyta or Cyanophyta)
= $10.0\ E_{480}$ (if species are predominately Chrysophyta or Pyrrophyta)

Interferences in the spectrophotometric determination of chlorophylls by the chlorophyll degradation products can be significant. Pigment samples from the aphotic zone, sediments, and samples from areas of high zooplankton grazing are particularly likely to contain these degradation products. Chemically these may consist predominantly of phaeophytin and phaeophorbide (phaeo-pigments) but sometimes large quantities of chlorophyllide may also be present. In the following procedure, it is possible to obtain a measure of the total quantity of chlorophyll *a* and phaeophytin *a* plus phaeophorbide *a*, only. Two similar procedures have been described for this determination (Moss 1967; Lorenzen 1967). This procedure employs equations in the second references.

After measuring the extinctions as above, two drops of dilute hydrochloric acid are added to the cuvette and mixed. Remeasure the extinction at 665 and 750 nm. Subtract each 750 nm reading from the corresponding 665 nm extinction and use the following equations to calculate the concentration of chlorophyll a and phaeo-pigments in the sample:

$$\text{Chl } a\ (mg/m^3) = \frac{26.7(665_o - 665_a)\,V_a}{V_w \times L}$$

$$\text{Phaeo-pigments (mg/m}^3\text{)} = \frac{26.7(1.7[665_n] - 665_o) \times V_a}{V_w \times L}$$

where 665_o is the extinction at 665 nm before acidification, 665_a the extinction at 665 nm after acidification, V_a is the volume of acetone used for extraction (mL), V_w the volume of water filtered (liters), and L the path length of the cuvette (cm).

CHAPTER 7.
SNOW

Snow is an important repository for airborne particulates and contaminants. The extent and quality of snow cover around stations is a fundamental landscape feature. The physical appearance and distribution of the snow cover are discussed in Chapter 5. In addition, discrete measurements of certain snow properties are deemed important in understanding the distribution and sources of disturbances around scientific stations in Antarctic. It is expected that the first indication of significant airborne pollution will be the accumulation of these materials in the adjacent snow-fields. Snow melt and the development of streams can also contribute contaminants to adjacent marine environments. For these reasons, discrete analyses of snow samples are limited to known contaminants that might be expected to be found in snow including particulates, trace metals, and hydrocarbons. Atmospheric transport of metals and hydrocarbons in emissions related to power generation and vehicular traffic are of interest. In most cases, the analysis of snow is the same as for liquid waters and where appropriate, the methods for preparing water samples for analysis are referred to. In addition, once the analytes of interest are isolated from the matrix, the instrumental analyses are often the same for all matrices (i.e., metals and hydrocarbons in soils). In these cases the methods are not repeated and the analyst is referred to the relevant sections.

Snow sampling presents some unique challenges and problems. In many instances the contaminant levels expected, other than in direct contact with emission sources, is low challenging the detection limits of even the most sophisticated instruments. The low concentrations expected also make snow samples subject to artifacts due to contamination during sampling, storage, and/or transport. For comparing data on snow, consistency in methodologies in regard to depth of sampling and normalization of data must be addressed as well. Various issues and approaches to sampling of snow have been discussed in Chapter 3 and will only be briefly repeated here.

Sampling of a snow field usually requires some type of grid sampling. As mentioned previously, sampling devices have to be scrupulously cleaned and all sample containers need to be ultra-clean. Solvents and other types of cleaning solutions are used to clean scrapers and other snow sampling devices. It is also recommended that polyethylene gloves, clean suits, and particle masks be used by sample collectors for the most sensitive sampling activities. Snow density kits can be used to collect basic snow accumulation. Metal and plastic shovels are recommended for excavating sites. Snowpit excavating and sampling has been previously described. Templates are used to ensure that the same depth and area of snow are sampled. Snow pack depth can be measured by penetration of snow probes or aluminum poles marked for measurement, and is often important in interpreting the history of the snow-field and the origin and concentrations of deposited contaminants (see Chapter 5).

7.1 Metals

Once the snow is melted, the sample needs to be stored and preserved for analysis. The decision needs to be made how the data should be reported. In those cases where complete characterization of a sample is desired, the suspended material is separately analyzed. This is

accomplished by filtration and acid digestion of the suspended material. Metallic constituents in the acid digest are subsequently determined and the sum of the dissolved plus suspended concentrations provides the total concentrations. The sample should be filtered as soon as possible after collection and the filtrate immediately acidified. The filtrate is acidified and digested at a later time by the procedures listed below. The dissolved and suspended metals are then reported. The total sample may also be treated with acid without prior filtration to measure what is termed "total recoverable" concentrations. Sufficient concentrated HNO_3 to lower the pH to <2.0 is added. The sample is stored in polyethylene or glass containers until digestion. It is suggested that if mercury is to be measured, the analysis should be conducted within 30 days. For other metals the sample will be stable for up to six (6) months.

7.1.1 Sample Preparation

For the determination of suspended metals a representative volume of unpreserved sample is filtered through a 0.45 µm membrane filter. When considerable suspended material is present, as little as 100 mL of a well mixed sample is filtered:

- Record the volume filtered and transfer the membrane filter containing the insoluble material to a suitable container for storage.
- The filtrate is transferred to a suitable container and acidified for preservation by the addition of concentrated HNO_3 to lower the pH to <2.0. Later this filter and/or the suspended material is added to a 250 mL Griffin beaker and 3 mL concentrated HNO_3 is added.
- Cover the beaker with a watch glass and heat gently. The warm acid will soon dissolve the membrane. Increase the temperature of the hot plate and digest the material.
- When the acid has nearly evaporated, cool the beaker and watch glass and add another 3 mL of concentrated HNO_3.
- Cover and continue heating until the digestion is complete, generally indicated by a light colored digestate.
- Evaporate to near dryness (DO NOT BAKE), add 5 mL distilled HCl (1:1) and warm the beaker gently to dissolve any soluble material. (If the sample is to be analyzed by the furnace procedure, 1 mL of 1:1 distilled HNO_3 per 100 mL dilution should be substituted for the distilled 1:1 HCl).
- Wash down the watch glass and beaker walls with deionized distilled water and filter the sample to remove silicates and other insoluble material that could clog the atomizer.
- Adjust the volume to a predetermined value based on the expected concentrations of metals present. The volume will vary depending on the metal to be determined.
- The sample is now ready for analysis. Instrument procedures are described in Chapter 8. Concentrations are reported as "suspended."

7.1.1.1 Acid Digestion Procedure for Furnace Atomic Absorption (FAA) Analysis

The following method is used to prepare samples for Furnace Atmomic absorption analysis.

- Shake sample and transfer 100 mL of well-mixed sample to a 250-mL beaker, add 1 mL of (1:1) HNO_3 and 2 mL 30% H_2O_2 to the sample.
- Cover with watch glass or similar cover and heat on a steam bath or hot plate for 2 hours at 95ºC or until sample volume is reduced to between 25 and 50 mL, making certain sample does not boil.
- Cool sample and filter to remove insoluble material. [NOTE: In place of filtering, the sample, after dilution and mixing, may be centrifuged or allowed to settle by gravity overnight to remove insoluble material].
- Adjust sample volume to 100 mL with deionized distilled water.
- The sample is now ready for analysis. Concentrations are reported as "total".

7.1.1.2 Acid Digestion Procedure for ICP and Flame AA Analyses

The following procedure is used to prepare samples for ICP and AA analysis.

- Shake sample and transfer 100 mL of well-mixed sample to a 250-mL beaker, add 2 mL of (1:1) HNO_3 and 10 mL of (1:1) HCl to the sample.
- Cover with watch glass or similar cover and heat on a steam bath or hot plate for 2 hours at 95ºC or until sample volume is reduced to between 25 and 50 mL, making certain sample does not boil.
- Cool sample and filter to remove insoluble material. [NOTE: In place of filtering, the sample, after dilution and mixing, may be centrifuged or allowed to settle by gravity overnight to remove insoluble material].
- Adjust sample volume to 100 mL with deionized distilled water.
- The sample is now ready for analysis. Concentrations are reported as "total".

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- **250 mL beaker** or other appropriate vessel
- **Watch glasses**
- **Thermometer** that covers range of 0 to 200ºC
- **Whatman No. 42 filter paper** or equivalent
- **ASTM Type II water (ASTM D1193):** Water must be monitored.
- **Concentrated nitric acid** (sp. gr. 1.41)
- **Concentrated hydrochloric acid** (sp. gr. 1.19)

7.1.1.3 Total Metals Sample Preparation Using Microwave Digestion

This method is an acid digestion procedure using microwave energy to prepare water and soil samples for analysis by GFAAS, ICP, or Flame AA for the following metals: aluminum, chromium, potassium, antimony, cobalt, selenium, arsenic, copper, silver, barium, iron, sodium,

beryllium, lead, thallium, cadmium, magnesium, vanadium, calcium, manganese, zinc, and nickel.

A representative 45 mL water sample is digested in 5 mL of concentrated nitric acid in a Teflon(R) PFA vessel or equivalent for 20 minutes using microwave heating. The digestate is then filtered to remove insoluble material. The sample may be centrifuged or allowed to settle by gravity overnight to remove insoluble material.

The water sample digestion procedure:

- A 45 mL aliquot of the sample is measured into Teflon(R) digestion vessels or equivalent using volumetric glassware.
- 5 mL of high purity concentrated HNO_3 is added to the digestion vessels.
- The caps with the pressure release valves are placed on the vessels hand tight and then tightened, using constant torque, to 12 ft./lbs. The weight of each vessel is recorded to 0.02 g.
- Place 5 sample vessels in the carousel, evenly spaced around its periphery in the microwave unit. Venting tubes connect each sample vessel with a collection vessel. Each sample vessel is attached to a clean, double-ported vessel to collect any sample expelled from the sample vessel in the event of over pressurization. Assembly of the vessels into the carousel may be done inside or outside the microwave.
- This procedure is energy balanced for five 45 mL water samples (each with 5 mL of acid) to produce consistent conditions. When fewer than 5 samples are digested, the remaining vessels must be filled with 45 mL of tap, DI or Type II water and 5 mL of concentrated nitric acid. Newer microwave ovens may be capable of higher power settings which may allow a larger number of samples. If the analyst wishes to digest more than 5 samples at a time, the analyst may use different power settings as long as they result in the same time temperature conditions defined in the power programming for this method. The initial temperature of the samples should be 24 ±1ºC. The preparation blank must have 45 mL of deionized water and the same amount (5 mL) of acid that is added to the samples. The microwave unit first-stage program must be set to give 545 watts for 10 minutes and the second-stage program to give 344 watts for 10 minutes. This sequence brings the samples to 160 ± 4ºC in ten minutes and permits a slow rise to 165-170ºC during the second 10 minutes.
- Following the 20 minute program, the samples are left to cool in the microwave unit for five minutes, with the exhaust fan ON. The samples and/or carousel may then be removed from the microwave unit. Before opening the vessels, let cool until they are no longer hot to the touch.
- After the sample vessel has cooled, weigh the sample vessel and compare to the initial weight as reported in the preparation log. Any sample vessel exhibiting a </= 0.5 g loss must have any excess sample from the associated collection vessel added to the original sample vessel before proceeding with

the sample preparation. Any sample vessel exhibiting a > 0.5 g loss must be identified in the preparation log and the sample redigested.

- Sample Filtration: The digested samples are shaken well to mix in any condensate within the digestion vessel before being opened. The digestates are then filtered into 50 mL glass volumetric flasks through ultra-clean filter paper and diluted to 50 mL (if necessary). The samples are now ready for analysis. The sample results must be corrected by a factor of 1.11 in order to report final concentration values based on an initial volume of 45 mL. Concentrations so determined shall be reported as "total".

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Note: Commercial kitchen or home-use microwave ovens shall not be used for the digestion of samples. The oven cavity must be corrosion resistant and well ventilated. All electronics must be protected against corrosion for safe operation.
Microwave oven with programmable power settings up to at least 600 watts.
The system must use PFA Teflon(R) digestion vessels (120 mL capacity) capable of withstanding pressures of up to 110 ± 10 psi (7.5 ± 0.7 atm). These vessels are capable of controlled pressure relief at pressures exceeding 110 psi.
A rotating turntable must be used to ensure homogeneous distribution of microwave radiation within the oven. The speed of the turntable must be a minimum of 3 rpm.
Polymeric volumetric labware in plastic (Teflon(R) or polyethylene) 50 mL or 100 mL capacity.
Whatman No. 41 filter paper (or equivalent).
Disposable polypropylene filter funnel.
Analytical balance: 300 g capacity, and minimum ± 0.01 g.
Polyethylene bottles: 125 mL, with caps.
ASTM Type II water (ASTM D1193): water must be monitored.
Sub-boiled, concentrated nitric acid (sp. gr. 1.41).
Concentrated hydrochloric acid (sp. gr. 1.19).

7.2 Total Petroleum Hydrocarbons (TPH)

Snow is collected in precleaned amber bottles, melted, and preserved by adding enough acid (HCl) to reduce the pH to <2. Alternatively, samples may be preserved by adding methylene chloride at a volume ratio of 1:40 methylene chloride:sample water. Unextracted samples and sample extracts are stored in the dark at or below 4°C. Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to false positives. All materials used in this method should be routinely demonstrated to be free from interferences by processing procedural blanks identical to samples (one blank per 20 samples or each batch, or whichever is more frequent). Matrix interferences result from the co-extraction of compounds other than the analytes of interest. Previous analyses indicate that matrix interferences are generally low. Therefore, silica column purification is only used in sample processing when indications such as particulates or color in the extract suggest that matrix interferences need to be removed. "Travel" and "field blanks' are a recommended part of the protocol due to the low-levels expected.

7.2.1 Sample Preparation

Glassware is cleaned by detergent (micro cleaning solution) washing with water and rinsing with tap water. Grease, often a hydrocarbon derivative, should not be used anywhere in these procedures. The glassware is then combusted in a muffle furnace at 400°C for at least 4 hours. Solvent rinses of acetone to dry followed by methylene chloride may be substituted for the muffle furnace heating when determined to be appropriate by the analyst. After drying and cooling, glassware is sealed and stored in a clean environment to prevent the accumulation of dust or other contaminants. Stored glassware is maintained capped with combusted aluminum foil.

"Apparatus, Materials, and Reagents - Total Petroleum Hydrocarbons"

- **Separatory Funnel**: 2 or 3-L Pyrex with Teflon stopcock.
- **Drying Column**: 20-mm ID Pyrex chromatographic column with glass wool at bottom and Teflon Stopcock, or Pyrex glass funnel.
- **Concentrator Tube**: Kuderna-Danish - 25 mL, graduated. Ground glass stoppers are used to prevent evaporation of extracts.
- **Snyder Column**: Kuderna-Danish - Three ball column.
- **Evaporative Flask**: Kuderna-Danish - 250 and 500 mL flat bottom flask.
- **Chromatographic Column**: 300-mm x 10-mm ID, with Pyrex glass wool at bottom and Teflon stopcock.
- **Water Bath**: heated to 60-70°C.
- **Graduated Cylinder**: 1 or 2 L.
- **Disposable Glass Pasteur Pipettes**: 1-mL.
- **Syringes**: 10 or 25 µL.
- **Teflon Boiling Chips**: Solvent extracted.
- **Vials**: 1-mL to 7-mL glass vials with Teflon-lined caps.
- **Nitrogen Gas Evaporation Unit**

Note: Volumetric glassware for sample measurement or introduction of internal standards must be calibrated.

- **Reagent Water**: Reagent water contains no analytes above the method detection limit.
- **Sodium Sulfate**: (ACS) Granular, anhydrous (purified by heating at 400°C for 4 hr in a shallow tray, or other suitable method).
- **Solvents**: Acetone or methanol (for rinsing), methylene chloride (pesticide quality or equivalent).
- **Hydrochloric Acid**: 6N
- **Silica gel**: 60-200 mesh, Davidson Grade 950 or equivalent. Should contain 1-2% water as defined by residue test at 130ºC. Adjust by overnight equilibration if needed.

7.2.2 Sample Processing

To prepare snow samples for analysis:

- Melt the snow to liquid water.

- Mark the sample bottle at the water meniscus for later determination of sample volume.
- If the sample was not acidified at time of collection, add 5 mL of hydrochloric acid to the sample bottle.
- After mixing the sample, check the pH by touching pH-sensitive paper to the cap to insure that the pH is 2 or lower.
- Add more acid if necessary. Pour the sample into a separatory funnel.
- Add 30 mL of methylene chloride to the sample bottle and rotate the bottle to rinse the sides.
- Transfer the solvent into the separatory funnel. Extract by shaking vigorously for 2 minutes.
- Allow the layers to separate.
- Filter the solvent layer through a funnel containing solvent moistened filter paper into a 125 mL flask.
- Any emulsion that fails to dissipate can be broken by pouring about 1 g of sodium sulfate into the filter paper cone and slowly draining the emulsion through the salt. Additional 1 g portions can be added to the cone as required.
- Repeat the extraction step twice more with 30 mL portions of fresh solvent, combining all solvent into the boiling flask.
- Rinse the tip of the separatory funnel, filter paper, and the funnel with a total of 5-10 mL solvent and collect the rinsings in the flask.
- Concentrate and exchange samples to 1 mL hexane.
- Prepare a packed glass column by adding 15 g silica on top of glass wool.
- Add the sample to top of the silica and elute with 100 mL methylene chloride into a boiling flask.
- Connect the boiling flask to the distilling head and evaporate the solvent by immersing the lower half of the flask in 70°C water.
- A solvent blank should accompany each set of samples.
- When the temperature in the distilling head reaches 50°C or the flask appears dry remove the boiling flask.
- Add a small amount of methylene chloride to boiling flask and volumetrically transfer the rinsate to a precleaned 10 dram bottle. Repeat 2 times.
- Blow dry the sample in the 10 dram bottle using dried nitrogen.
- Bring the volume of the 10 dram bottle to exactly 1 mL with methylene chloride.
- Accurately weigh a 20 µL aliquot of this 1 mL sample on an electrobalance.

7.2.3 Calculations

Calculate the petroleum hydrocarbons in the sample using the formula:

$$\text{mg / L total petroleum hydrocarbons} = \frac{\text{A x } \frac{V_1}{V_2}}{V_3}$$

where:

A = weight of aliquot (mg)
V_1 = total volume of extract (usually 1000 µL)
V_2 = volume of extract weighed (usually 20 µL)
V_3 = volume of water extracted (usually 1 L)

Once the extract is obtained, hydrocarbons can be determined by the range of instrumental methods described in Chapter 8.

7.3 Particulates

This method estimates the amount of particulate matter in snow. The size of the aerosol particles found in the snow extends from several microns to well below 0.1 µm based on electron scanning micrographs of a nucleopore filter samples.

7.3.1 Sample Preparation and Processing

Collect **s**now samples using a plastic spatula to scrape fresh snow into 500 mL polyethylene jars as described in Chapter 3. Plastic gloves and nylon outer clothing should be worn to reduce the likelihood of sample contamination. Samples must be collected facing into the wind to minimize contamination. Containers should be sealed tightly and placed into plastic bags for transport to the laboratory. Keep samples frozen during transport to reduce adsorption of soot onto container walls and to reduce effects due to algae growth or chemical reactions.

"Apparatus, Materials, and Reagents - Particulate Measurements"

- **Preweighed quartz membranes**.
- **Microwave**
- **Nucleopore filter**: 25 mm, 0.4 µm pore size
- **Pore Filter**: 0.45-tan pore for backup.
- **Aluminum oxide**: 3 suspensions of aluminum oxide, each having approximately 3mg L^{-1} of aluminum oxide particles, with mean diameters of 0.05, 0.3, and 1.0 µm.
- **Hydrosol**: Prepare a 100 mg of Monarch 71(M71) calibration soot (Cabot Corporation) by dissolving in 80 mL filtered water and 20 mL isopropyl alcohol. Disperse soot particles by then placing the hydrosol in an ultrasonic bath for several minutes. Then prefilter hydrosol through a 2.0 µm pore diameter Nucleopore filter to produce soot particles in the same size range as found in atmospheric aerosols. Next, prepare three aliquots of a 1/10 solution of the stock hydrosol with one of these saved as a reference. Then, one half of the second aliquot needs to be diluted for use in subsequent steps. Filter the third through one of the prepared Nucleopore filters.

The particulate matter in snow can be determined by filtering melt water through preweighed quartz filters membranes. Combust quartz membranes at 80ºC before use to remove all organic binders and contaminants. Preweigh the membranes before use. Microwave snow

samples and extract within a minute of actual snowmelt to minimize losses of particulates to walls of the sample container. Filter the particulates from the melt water. Compare the difference in weights from the preweighed and dried membranes to determine the concentration of particulate matter in the sample.

CHAPTER 8.
SOILS/SEDIMENTS

Soils and sediments are often the ultimate repository of contaminants released to the environment. Soils and sediments also provide the substrate that supports the associated biological communities. Changes in soil and sediment characteristics can in and of themselves cause significant changes in biological communities. In addition to direct physical/mechanical disturbance, sediments and soils are also a major pathway for exposure of biological organisms to contaminants. As such, the first tier of parameters of interest to Antarctic monitoring programs include basic soil/sediment characteristics (i.e., grain size and carbon content) and quantitative determinations of contaminant distributions (i.e., metals and hydrocarbons). The determination of the concentration and distribution of solid-associated contaminants is important in understanding changes in biological communities. These matrices tend to serve as a repository of contaminants that may accumulate over long periods of time and can serve as a source of exposure long after the initial discharge or release event has occurred. Various processes also contribute to a continued redistribution of the more persistent contaminants throughout the system being monitored over time.

For monitoring programs in Antarctica the importance or need to monitor soils and sediments will be dependent on the environmental setting of the station. In some areas soils may not be exposed because they are permanently covered with snow. In these cases the snow itself would be the repository for contaminants and soils may not need to be monitored. In situations where soil is routinely exposed at the site for some period of the year, soils will be an important medium to monitor to determine if significant contamination is accumulating in the area. Sediments can be from freshwater or marine settings. If there are significant accumulations of water in close proximity to the station then there is potential for human activities to generate contaminants that will directly or indirectly make their way into nearby water bodies. The most common contaminants associated with the presence of humans in Antarctica are petroleum hydrocarbons and metals. In special cases other chemicals may have been released to the area such as pesticides and polychlorinated biphenyls (PCBs) due to practices in the past. While mostly discontinued, incineration also introduces hydrocarbons as aerosols in the emissions. The extent and stability of contaminants in the environs around a station are an important consideration for program managers. Again, a complete understanding of the activities at the station and the identification of potential impacts will guide the development of the monitoring program and dictate which matrices and parameters are important.

8.1 Grain Size

Sediment texture is an important variable in the evaluation of contaminant concentrations and biological patterns in monitoring programs. Numerous studies have shown a correlation between contaminant concentration and grain size. In benthic ecosystem studies, biological patterns are often dependent on substrate characteristics.

"Apparatus, Materials, and Reagents - Grain Size"

- **Large mason jars or equivalent**: 1 pint.
- **Beakers**: 50 mL.

- **Graduated cylinders**: 1-liter.
- **Analytical balance**: 0.1 mg accuracy.
- **Pipette**: 20-mL capacity.
- **Drying oven**: Maintained at 40°-50°C and 100°-130°C.
- **Timer**: 1 second intervals.
- **Shaker table**
- **Sodium hexametaphosphate solution**: concentration: 5.5 g/L.
- **Distilled water**
- **Hydrogen Peroxide:** 30%
- **62.5 micron screen**

The most common method for the analysis of silt and clay sized particles is the pipette method (Folk 1974). It is based on the settling velocity of the particles computed on the basis of Stokes' Law. At given times, small volumes of suspension are withdrawn, evaporated, and the residue weighed.

8.1.1 Sample Preparation

Sediments are collected in glass jars, core liners or plastic bags by appropriate sampling techniques (i.e., scoop, grab or boxcorer, diver, etc). The preferred container is a plastic bag. At a minimum, 50 g of sample is placed in a plastic bag, sealed and labeled. Samples should be refrigerated, not frozen (4ºC). Duplicate samples are analyzed for every 20 samples.

To prepare the sample for processing:

- Homogenize the sample (massage sample bag by hand).
- Place ~15-20 grams of sample in a large glass jar. This sample size is chosen to minimize the interference of grains with each other during settling and the possibility of flocculation as well as maximize the amount of material to be weighed (i.e., with small samples the error in weighing becomes large with respect to the sample weight).
- Treat the sample with ~50-100 mL of 30% hydrogen peroxide (volume varies with the amount of organic matter present) for 12 hours prior to analysis to oxidize organic matter.
- Wash the sample with distilled water to remove all soluble salts.
- Four hundred (400) mL of sodium hexametaphosphate solution (~5.5 g/L) is added to disperse the sample, followed by shaking for ~24 hours on a shaker table.

A 62.5 micron screen is placed over a 1-liter graduated cylinder. The solution containing the dispersed sediment is poured over the screen and washed with dispersant to rinse any remaining fine-grained sediment into the cylinder. This separates the gravel/sand fraction (on the screen) from the silt/clay fraction (in the cylinder).

8.1.2 Sample Processing

Samples are then further processed:

- Wash the coarse fraction into a preweighed beaker with distilled water.
- Place the beaker in an oven (100°-130°C) for 24 hours to dry.
- Remove the beaker from the oven and leave to cool to room temperature.
- Leave the beaker open in the room where weighing is to take place for several hours to allow equilibration with the atmospheric moisture.
- Weigh the beaker to 0.1 mg with an analytical balance.
- If both sand and gravel are required, the sand fraction is dry sieved at 2 mm (-1.0 phi) and 62.5 micron (4 phi) intervals to separate gravel from sand-sized material.
- The weights of the gravel (>2 mm) and sand-sized (62.5 micron to 2 mm) material are recorded.

The graduated cylinder containing the silt/clay material is filled to exactly one liter volume with sodium hexametaphosphate dispersant solution. Then,

- The cylinder is stirred vigorously and left to stand for one (l) day.
- If the cylinder shows no sign of flocculation, analyses may proceed. If flocculation is apparent, the sample will have to be discarded and the procedure restarted.
- The fine fraction is analyzed at 4 phi and 8 phi intervals.
- Two labeled beakers are preweighed to 0.1 mg.
- The cylinder is stirred vigorously starting at the bottom and working up until all the sediment is distributed uniformly throughout the cylinder.
- At the end of the vigorous stirring, long smooth strokes are used for the full length of the cylinder (from the bottom until the stirring rod breaks the surface).
- As soon as the rod emerges for the last time, start the timer.
- Insert the pipette to a depth of 20 cm, and at the end of 20 seconds, withdraw exactly 20 mL (this is the 4 phi aliquot). This is the most important single step, because all subsequent analysis are based on this weight (the dry weight of the 4 phi aliquot is the total weight of the silt + clay fraction).
- Pipette the suspension into a preweighed beaker, rinse the pipette with 20 mL of distilled water, and add the rinse water to the same beaker.
- At the 2:03:00 (two hours, 3 minutes) time, withdraw a 20 mL aliquot at a depth of 10 cm; this is the 8 phi aliquot.
- Pipette the suspension into a different preweighed beaker, rinse with 20 mL of distilled water and add to the beaker.
- The beakers are placed in an oven and evaporated to dryness for at least 24 hours at 100-130°C.
- After 24 hours, they are removed from the oven and left to cool to room temperature. They are left open in the room where weighing is to take place for several hours, so that they can come to equilibrium with the atmospheric moisture.
- The beakers are weighed to 0.1 mg with an analytical balance, and the weights recorded.

8.1.3 Calculations

Grain-size data is normally reported in phi intervals rather than in microns, millimeters, or inches. The phi (ϕ) diameter is computed by taking the negative log of the diameter in millimeters. Statistical computations and graphic presentations are much simpler when phi diameters are used. The table below summarized the phi distribution, two microns are sometimes used as the silt/clay boundary.

phi	Diameter (in mm)	Class	Grade
-8	256	gravel	boulder
-6	64	gravel	cobble
-2	4	gravel	pebble
-1	2	gravel	granule
0	1	sand	very coarse
1	0.5	sand	coarse
2	0.25	sand	medium
3	0.125	sand	fine
4	0.0625	sand	very fine
5	0.313	silt	coarse
6	0.0156	silt	medium
7	0.0078	silt	fine
8	0.0039	silt	very fine
9	<0.0039	clay	

The 4 and 8 phi dry weights include the weight of the added dispersant. Multiply the dispersant weight (grams per 1 liter used = 5.5 g) by the fraction of the total solution removed (20/1000) and subtract from the aliquot weight. This total is then multiplied by 50 (1000 mL/20 mL) to yield the sample weight of the silt + clay fraction. Three weights are needed to calculate the total dry sample weight. All weights are in grams.

- wt. sand (2 mm to 62.5 micron size range) + wt. gravel (2 mm and greater size range) + wt. of 4 phi residue = total dry sample weight
- % gravel = wt. gravel fraction / total dry sample wt.
- % sand = wt. sand fraction / total dry sample wt.
- % silt = [(wt. 8 phi residue - dispersant) x 50] / total dry sample wt.
- % clay = {[(wt. 4 phi-wt. 8 phi) - dispersant] x 50} / total dry sample wt.

The reporting units for grain size are percent gravel, sand, silt and clay on a weight basis. The minimum method performance standard for the method is detection of 0.5 percent of each fraction. Results are reported to three (3) significant figures. All duplicate analyses are reported. Duplicate analyses are analyzed at a frequency of at least every 20 samples.

8.2 Carbon Content

The carbon content of soils and sediments is a fundamental property. Carbon can be both organic and inorganic in origin. Organic carbon is often the source of energy that supports the microbiological community at the base of the food chain. Additions of labile carbon can also contribute to eutrophication serving as a fuel for aerobic degradation and oxygen consumption.

Anoxic conditions have a strong influence on biological patterns. Inorganic carbon content and composition is often related to local mineralogy (carbonates) and the presence of organisms that develop carbonate testes and shells. Soils and sediment are also a repository of contaminants with organic carbon content often determining the capacity of the sediment to adsorb and retain contaminants. The carbon content of soils and sediments, and its distribution between organic and inorganic species, is important in understanding the origins and changes in soils and sediments in the area being monitored.

The following procedures are used to determine the total carbon, total organic carbon, and total inorganic carbon content of soils/sediments. Carbon concentrations are determined on dried sediment/soil using a LECO Model 523-300 induction furnace or equivalent, an infrared (IR) detector, and an integrator. Other commercially available carbon analyzers produce comparable results.

"Apparatus, Materials, and Reagents - Carbon Content"

- **Mortar and pestle**: 500-mL mortar or other suitable container.
- **Forceps**.
- **LECO Model 523-300 Induction Furnace** or equivalent.
- **Horiba PIR-2000 Infrared detector** or other suitable detector.
- **HP 3390A Integrator** or other suitable recorder/integrator.
- **Rotometer**.
- **Flow controller**.
- **Glass measuring scoop**.
- **Analytical balance** capable of weighing to 1 mg.
- **10% HCl** (V:V).
- **Platinized silica**.
- **Anhydrone** (magnesium perchlorate).
- **Manganese dioxide**.
- **HPLC grade reagent water**.
- **Iron chip accelerator**.
- **Copper metal accelerator**.
- **Combustion crucibles**.
- **Pin and ring carbon standards**: range: 0.1 to 1.0% carbon.

Samples are combusted in an oxygen atmosphere in the furnace and the combustion gases produced are swept out of the combustion chamber by the oxygen flow. The combustion gases pass through two reaction tubes in series and a gas filter tube to remove any particles before entering the detector. The first reaction tube is a two-stage chamber. The first stage contains manganese dioxide which absorbs only sulfur oxides that may have formed during combustion. The second stage contains anhydrone which removes water vapor from the gas stream. The second reaction tube, filled with platinized silica, is maintained at an elevated temperature by an external heating sleeve. The contents of this tube act as a catalyst to convert carbon monoxide to carbon dioxide. The carbon dioxide is detected using a Horiba PIR-2000 infrared detector or equivalent. The output signal from the Horiba is sent to an integrator, which processes the signal. The resulting signal is then converted to % carbon content based upon a calibration curve established at the beginning of the analysis. Total carbon (TC) is determined on an unacidified

dry sample, while total organic carbon (TOC) is determined after sample acidification. Total inorganic carbon (TIC) is calculated as the difference between TC and TOC.

Sediments are collected in glass jars, core liner, or plastic bags by appropriate sampling techniques (scoop, grab or boxcorer, diver, etc.). Soils/sediments are stored at -20°C ±10°C until analysis. After sub-sampling, excess sample is archived at -20°C ±10°C in the dark.

8.2.1 Total Carbon (TC)

For the analysis of total carbon in soils or sediments:

- One hundred fifty (150) to 300 mg (to the nearest milligram) of dried, finely ground, homogenized sediment/soil are weighed into a tared, carbon-free combustion crucible. The amount of sample depends on the expected carbon concentration.
- One scoop each of copper and iron chip accelerants is added to the sample crucibles prior to combustion.
- All sample crucibles and QC sample crucibles are kept covered with aluminum foil until analysis.
- The calibration blank and standards are prepared.
- Blank and calibration standard are not acidified or oven dried.
- The blank is an empty crucible with one scoop of copper and iron chip accelerant.
- Check standards are required for routine analytical activities. Use pin or ring carbon mid-level carbon standards.
- The appropriate carbon ring or pin calibration standard is placed in the crucible and one scoop each of copper and iron chip accelerant is added.
- The method detection limit (MDL) standard uses a pin or ring low-level carbon standard with a carbon content of 0.03%. Other low-level carbon standards may be used.
- The appropriate carbon ring or pin calibration standard is placed in the crucible and one scoop each of copper and iron chip accelerant is added.
- The non-certified TOC content of the NIST SRM 1941a is 4.8% ± 1.2% on a dry weight basis (other SRMs may be used). 0.080 grams (to the nearest milligram) of this SRM is weighed in a tared, carbon-free combustion crucible. The amount of the SRM depends on the carbon content, which is between 3.6 to 6.0% as TOC.
- One scoop each of copper and iron chip accelerant is added.
- A duplicate sample is selected at random and prepared accordingly.
- After mixing, a second aliquot of approximately the same weight is prepared from the finely ground, homogenized, dry sediment/soil sample.
- One scoop each of copper and iron chip accelerant is added.
- The crucible is placed on the oven pedestal using forceps.
- The oven is closed and oxygen flow is started.
- The oxygen is allowed to flow for 15 seconds and the flow rate on the rotometer is checked and adjusted to 90 as needed.

- After 15 seconds with the correct flow, the integrator is started and pedestal lever is pushed in to start the induction furnace.
- Approximately 20 seconds after the furnace is activated, the carbon begins to combust.
- About one minute after turning the furnace on, the ammeter will reach 500 mA.
- When the signal returns to a baseline value, the furnace is turned off.
- All calibration and sample analyses are recorded.

8.2.2 Total Organic Carbon (TOC)

For the analysis of soils and/or sediments for TOC:

- An appropriate amount of dried sample and duplicate sample is weighed and prepared in a tared crucible as described above.
- Small amounts of 10% HCl solution are slowly added to the sample until all bubbling stops. Use the smallest volume of acid possible.
- The treated samples are dried overnight at 50°C in a drying oven.
- One scoop each of copper and iron chip accelerants is added to all of the sample crucibles.
- The above procedures are followed for combusting the TOC samples.
- The calibration blank and standards are prepared in the same manner. The laboratory blank is added to an empty crucible which has been acidified and placed in the 50ºC oven overnight.
- One scoop each of copper and iron chip accelerant is added.
- Check standards are standards with a mid-level carbon content. These standards are not acidified.
- The appropriate carbon ring or pin calibration standard is placed in the crucible and one scoop each of copper and iron chip accelerant is added.
- The method detection limit (MDL) standard uses a low-level carbon standard between 0.03 and 0.07%. Other low-level carbon standards may be used. These standards are not acidified.
- The appropriate carbon calibration standard is placed in the crucible and one scoop each of copper and iron chip accelerant is added.
- The non-certified TOC content of the NIST SRM 1941a is 4.8 ± 1.2 % on a dry weight basis.
- 0.080 grams (to the nearest milligram) of this SRM is weighed in a tared, carbon-free combustion crucible.
- The SRM is acidified and oven dried as described above. One scoop each of copper and iron chip accelerant is added.
- The duplicate sample is selected and prepared at random.
- A second aliquot of approximately the same weight is prepared from the finely ground, homogenized, dry sediment/soil sample.
- The duplicate described above is acidified and oven dried.
- One scoop each of copper and iron chip accelerant is added. The crucibles used for these blanks are not acidified or oven dried.

- The calibration samples, the acidified samples, and associated QC samples are analyzed as described above.
- All data regarding calibration activities and sample analyses are recorded.

8.2.3 Total Inorganic Carbon (TIC)

The % total inorganic carbon (TIC) content is determined by subtracting the % total organic carbon (TOC) from the % total carbon (%TC) content.

The following is an example analytical sequence.

Activity	QC Criteria
Calibrate; blank plus four standards	r^2 □0.995
Check Standard (Mid-Level)	± 10% of true value
SRM 1941a	± 25% of true (3.6 -6.0% C)
MDL standard (approx. 0.014-0.029% C)	± 25% of true value
1. Prep. Blank	□3xMDL
2. Orig. Sample (□0.3 gram)	Within Calibration Range
3. Duplicate Sample (□0.3 g)	RPD □25% Low % (<1%) %C
	RPD □20% High %(>1%) C
4. to 10. Samples (□0.3 g)	Within Calibration Range
Check Standard (Mid-Level)	± 15% of true value
11. to 18. samples (□0.3 g)	Within Calibration Range
Check Standard (Mid-Level)	± 15% of true value

8.2.4 Calculations

Quality control samples are prepared and analyzed in an identical manner to the actual samples. A five point initial calibration curve is prepared each day prior to analyzing samples. The QC acceptance criteria for the initial calibration curve is a correlation coefficient □ 0.995. Analytical results determined by this procedure must be within the calibration range established for the calibration curve. If not, sample size is reduced or the calibration range extended. Mid-level standards with carbon are analyzed after the calibration curve has been established. Check standards are also analyzed after every 10 samples (or with every sample set, whichever is more frequent). A check standard is also analyzed at the end of the analytical sequence as a check. The QC acceptance criteria for the check standard is ± 15% of the known value. If the check standard fails this criteria, the analyses are stopped and the instrument recalibrated. A laboratory blank is analyzed with every 20 samples, or with every sample set, whichever is more frequent. The QC acceptance criteria for carbon in the blank is less than or equal to three times the detection limit (□0.06%). If this QC criteria is not met, all samples must be re-prepared and re-analyzed. Duplicate samples are analyzed every 20 samples, or with every sample set, whichever is more frequent. Except as noted below, the QC acceptance criteria for the RPD for duplicates is an RPD of ±25% for low level (<1.0% carbon) samples and □20% for high level (>1.0% carbon) samples. If this QC criteria is not met, all samples must be re-prepared and re-analyzed unless the samples are inhomogeneous. A higher RPD is acceptable for inhomogeneous samples (i.e., peat, sandy loams, grasses, shell hash, etc.). The non-certified TOC content of the NIST SRM 1941a is 4.8 ± 1.2 % on a dry weight basis (other SRMs may be used). This SRM is analyzed for every 20 samples and the QC acceptance criteria for the carbon content of SRM 1941a is between 3.6 to 6.0 % TOC. This is equivalent to a percent recovery of

75 to 125% (± 25% of the known value). If an SRM fails the QC criterion, the instrument must be recalibrated, a check standard analyzed, and all samples are re-analyzed. A MDL standard containing between 0.03 and 0.07% carbon is analyzed with every 20 samples, or with every sample set, whichever is more frequent. Other low-level carbon standards may be used. The QC acceptance criteria for carbon levels in the MDL standard is ± 25% of the known value for the standard used. If the MDL standard analysis fails the criterion, the instrument must be recalibrated, a check standard analyzed and all samples are re-analyzed.

Prior to sample combustion, a set of standards is analyzed to establish an initial calibration curve. All data regarding calibration and sample analyses are recorded.

To determine the calibration curve, combust one blank and four carbon standards at % carbon contents of approximately 0.03, 0.390, 0.652, and 0.835%. Other carbon standards can be used. The values of the standards in the set cover the 0 to 0.8% carbon calibration range (based on a sample weight of 1 gram). The calibration blank is an empty crucible containing one scoop each of copper and iron chip accelerators. The calibration blank is not acidified and is combusted prior to the carbon standards. The appropriate carbon ring or pin calibration standards are placed in the crucible and one scoop each of copper and iron chip accelerators is added prior to combustion. Calibration standards are not acidified.

A graphic representation of the calibration curve (integrator response vs. carbon percentage) is used to calculate the best fit equation for the data. The QC acceptance criterion for the calibration curve is that the correlation coefficient, r^2, should be ≥ 0.995. Calibration analyses are repeated until the QC criterion are met. The calibration curve is used to determine the carbon content of the samples analyzed.

8.3 Metals

The methods for metals often provide for the determination of a large number of metals with a single technique. Thus the metal methods are considered slightly different from the other methods in this manual. Since the same metal can be determined by multiple instrumental techniques it is most effective to describe these methods by technique. In many cases the methods provide substantially different detection limits and the method of choice will depend on the ambient levels expected in the matrix of interest. Certain methods, such as for mercury, have been specifically developed for the characteristics of the analyte of interest. Access to various types of instruments may also be limited and therefore the selection of the method is at the analyst's discretion. For the specific metals of interest in this first tier of methods the following methods are recommended: cadmium (Cd) and lead (Pb) - graphite furnace atomic absorption spectrophotometry (GFAAS), copper (Cu) and zinc (Zn) - inductively-coupled plasma emission spectroscopy (ICP), and mercury (Hg) - cold vapor atomic absorption spectroscopy (CVAAS). In each instance the protocols for all other metals that can be analyzed by the various techniques are also provided. As far as matrix, once the metals are converted to a soluble form, the instrumental method is usually the same and may only need to take into account differences in concentrations and/or interferences.

8.3.1 Sample Preparation

The choice of metals for a monitoring program are based on the potential sources in the area and the known potential to induce biological effects. Metals are a common pollutant released by human activities and they can be derived from a wide range of sources. Metals are derived from the disposal of various types of materials (metal debris, batteries, etc.), degeneration of structures, construction activities (such as welding), and from metallic plumbing systems. Metals are known to be toxic to biological organisms. However, the mere presence of a chemical, in and of itself, does not indicate that adverse biological effects have occurred. The availability of the metal and its geochemical behavior will ultimately determine the potential for exposure and whether an adverse effect occurs in an exposed biological community. Different organisms also have differing sublethal and toxic response thresholds to metals. As a first indication of the degree of contamination, establishment of the concentration and distribution of metals is important. The observed concentrations can be compared, at least qualitatively, to documented effects levels in order to make a first assessment of the potential for biological impacts. It should also be recognized that metals naturally occur in soils and sediments and that anthropogenic inputs are in addition to these natural levels. It is important to establish background levels within a study area so that increases due to human activity can be unambiguously recognized.

The metals of interest for Antarctic monitoring programs were chosen based on knowledge of the expected outputs from typical activities that occur at stations and the available metal information previously collected and include cadmium, lead, copper, zinc, and mercury. A brief description of suspected levels of biological effects is provided for each metal.

Studies have documented biological effects associated with cadmium levels of 5 mg/kg and higher (Long and Morgan 1990). Effects include mortality, reduced growth, inhibited reproduction, and other adverse effects. Most studies were of freshwater and marine invertebrates. Copper levels in sediments of 17.8 to 2820 mg/kg have been associated with biological effects. Zinc concentrations in sediments of 50 to 250 mg/kg are associated with sublethal biological effects and effects almost always occurred at zinc concentrations above 260 mg/kg. Biological effects have been observed for mercury sediment concentrations as low as 0.15 mg/kg. It should be noted that these threshold levels for biological effects are only advisory and actual metal concentrations in the field may or may not be reflective of these data.

8.3.1.1 Acid Digestion Procedure for ICP, Flame AA and Furnace AA Analyses

A representative 1 g (wet weight) sample is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed with either nitric acid or hydrochloric acid. Hydrochloric acid is used as the final reflux acid for the furnace AA analysis of Sb, the Flame AA or ICP analysis of Al, Sb, Ba, Be, Ca, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Ni, K, Ag, Na, Tl, V and Zn. Nitric acid is employed as the final reflux acid for the Furnace AA analysis of As, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Ag, Tl, V, and Zn.

The procedure is as follows:

- Mix the sample thoroughly to achieve homogeneity.

- For each digestion procedure, weigh (to the nearest 0.01g) a 1.0 to 1.5 g portion of sample and transfer to a beaker.
- Add 10 mL of 1:1 nitric acid (HNO_3), mix the slurry, and cover with a watch glass. Heat the sample to 95^{o}C and reflux for 10 minutes without boiling.
- Allow the sample to cool, add 5 mL of concentrated HNO_3, replace the watch glass, and reflux for 30 minutes. Do not allow the volume to be reduced to less than 5 mL while maintaining a covering of solution over the bottom of the beaker.
- After the second reflux step has been completed and the sample has cooled, add 2 mL of Type II water and 3 mL of 30% hydrogen peroxide (H_2O_2).
- Return the beaker to the hot plate for warming to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides, and cool the beaker.
- Continue to add 30% H_2O_2 in 1 mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged. [NOTE: Do not add more than a total of 10 mL 30% H_2O_2].
- If the sample is being prepared for the furnace AA analysis of Sb, the flame AA or ICP analysis of Al, Sb, Ba, Be, Ca, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Ni, K, Ag, Na, Tl, V, and Zn, add 5 mL of 1:1 HCl and 10 mL of Type II water, return the covered beaker to the hot plate, and heat for an additional 10 minutes.
- After cooling, filter through Whatman No. 42 filter paper (or equivalent) and dilute to 100 mL with Type II water. [NOTE: In place of filtering, the sample (after dilution and mixing) may be centrifuged or allowed to settle by gravity overnight to remove insoluble material. The diluted sample has an approximate acid concentration of 2.5% (v/v) HCl and 5% (v/v) HNO_3. Dilute the digestate 1:1 (200 mL final volume) with acidified water to maintain constant acid strength. The sample is now ready for analysis].
- If the sample is being prepared for the furnace analysis of As, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Ag, Tl, V, and Zn, continue heating the acid-peroxide digestate until the volume has been reduced to approximately 2 mL, add 10 mL of Type I water, and warm the mixture.
- After cooling, filter through Whatman No. 42 filter paper (or equivalent) and dilute the sample to 100 mL with Type I water (or centrifuge the sample). [NOTE: In place of filtering, the sample (after dilution and mixing) may be centrifuged or allowed to settle by gravity overnight to remove insoluble material. The diluted digestate solution contains approximately 2% (v/v) HNO_3. Dilute the digestate 1:1 (200 mL final volume) with acidified water to maintain constant acid strength. For analysis, withdraw aliquots of appropriate volume, and add any required reagent or matrix modifier. The sample is now ready for analysis.]

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- **250 mL beaker** or other appropriate vessel
- **Watch glasses**
- **Thermometer that covers range of 0 to 200ºC**
- **Whatman No. 42 filter paper or equivalent**
- **ASTM Type II water (ASTM D1193):** Water must be monitored
- **Concentrated nitric acid** (sp. gr. 1.41)
- **Concentrated hydrochloric acid** (sp. gr. 1.19)
- **Hydrogen Peroxide** (30%)

8.3.1.2 Total Metals Sample Preparation Using Microwave Digestion

A representative 0.5 g (wet weight) sample is digested in 10 mL of concentrated nitric acid in a Teflon(R) PFA vessel or equivalent for 10 minutes using microwave heating. The digestate is then filtered to remove insoluble material. The sample may be centrifuged or allowed to settle by gravity overnight to remove insoluble material. [NOTE: This microwave digestion method is not appropriate for the quantitative recovery of Antimony from soil and sediment samples.]

The calibration procedure is a critical step prior to the use of any microwave unit. The microwave unit must be calibrated every six months. The calibration data for each calibration must be available for review during on-site audits. In order to interchange the absolute power settings from one microwave unit to another, the actual delivered power must be determined.

Calibration of a laboratory microwave unit depends on the type of electronic system used by the manufacturer. If the unit has a precise and accurate linear relationship between the output power and the scale used in controlling the microwave unit, then the calibration can be a two-point calibration at maximum and 40% power. If the unit is not accurate or precise for some portion of the controlling scale, then a multiple-point calibration is necessary. If the unit power calibration needs a multiple-point calibration, then the point where linearity begins must be identified. For example: a calibration at 100, 99, 98, 97, 95, 90, 80, 70, 60, 50 and 40% power settings can be applied and the data plotted. The non-linear portion of the calibration curve can be excluded or restricted in use. Each percent is equivalent to approximately 5.5-6 watts and becomes the smallest unit of power that can be controlled. If 20-40 watts are contained from 99-100%, that portion of the microwave calibration is not controllable by 3-7 times that of the linear portion of the control scale and will prevent duplication of precise power conditions specified in that portion of the power scale.

The power available for heating is evaluated so that the absolute power setting (watts) may be compared from one microwave to another. This is accomplished by measuring the temperature rise in 1 Kg of water exposed to microwave radiation for a fixed period of time. The water is placed in a Teflon(R) beaker (or a beaker that is made of some other material that does not adsorb microwave energy) and stirred before measuring the temperature. Glass beakers adsorb microwave energy and may not be used. The initial temperature of the water must be between 19 and 25ºC. The beaker is circulated continuously through the field for at least two (2) minutes at full power. The beaker is removed from the microwave, the water is stirred

vigorously, and the final temperature recorded. The final reading is the maximum temperature reading after each energy exposure. These measurements must be accurate to ±0.1ºC and made within 30 seconds of the end of heating. If more measurements are needed, do not use the same water until it has cooled down to room temperature. Otherwise, use a fresh water sample.

The absorbed power is determined by the following formula:

$$P = [(K) \times (Cp) \times (m) \times (DT)] / t$$

where:

P = The apparent power absorbed by the sample in watts (joules per second),
K = The conversion factor for thermochemical calories per second to watts (-4.184),
Cp = The heat capacity, thermal capacity, or specific heat (cal. $g^{-1}ºC^{-1}$) of water (-1.0),
m = The mass of the sample in grams (g),
DT = the final temperature minus the initial temperature (ºC), and
t = the time in seconds (s)

Using 2 minutes and 1 Kg of distilled water, the calibration equation simplifies to:

$$P = (DT) (34.87).$$

The microwave user can now relate power in watts to the percent power setting of the microwave

The initial cleaning of the PFA vessels:

- Prior to first use - new vessels must be annealed before they are used. A pretreatment/cleaning procedure must be followed. This procedure calls for heating the vessels for 96 hours at 200ºC. The vessels must be disassembled during annealing and the sealing surfaces (the top of the vessel or its rim) must not be used to support the vessel during annealing.
- Rinse in ASTM Type I water.
- Immerse in 1:1 HCl for a minimum of 3 hours after the cleaning bath has reached a temperature just below boiling.
- Rinse in ASTM Type I water.
- Immerse in 1:1 HNO_3 for a minimum of 3 hours after the cleaning bath has reached a temperature just below boiling.
- The vessels are then rinsed with copious amounts of ASTM Type I water prior to use for any analyses under this contract.

Cleaning procedure between sample digestions

- Wash entire vessel in hot water using laboratory-grade nonphosphate detergent.
- Rinse with 1:1 nitric acid.

- Rinse three times with ASTM Type I water. If contaminants are found in the preparation blank, it is mandatory that steps a(2) through a(6) be strictly adhered to.

The digestion procedure is as follows:

- Add a representative 0.5 ±0.050 grams of sample to the Teflon(R) PFA vessel or equivalent.
- Add 10 ±0.1 mL of concentrated nitric acid. If a vigorous reaction occurs, allow the reaction to stop before capping the vessel.
- Cap the vessel, then tighten using constant torque to 12 ft/lbs, according to the manufacturer's direction.
- Connect the sample vessel to the overflow vessel using Teflon(R) PFA tubing or equivalent.
- Weigh the vessel assembly to the nearest 0.01g.
- Place sample vessels in groups of 2 sample vessels or 6 sample vessels in the carousel, evenly spaced around its periphery in the microwave unit. If fewer than the recommended number of samples are to be digested (i.e., 3 samples plus 1 blank) then the remaining vessels must be filled with 10 mL of nitric acid to achieve the full complement of vessels. Each sample vessel must be attached to a clean, double-ported vessel to collect any sample expelled from the sample vessel in the event of over pressurization. Assembly of the vessels into the carousel may be done inside or outside the microwave. Connect the overflow vessel to the center well of the oven.
- The preparation blank must have 0.5 mL of deionized water and the same amount (10 mL) of acid that is added to the samples. The preparation blank must later be diluted to 50 mL in the same manner as the samples.
- Irradiate the 2 sample vessel group at 344 watts for 10 minutes, or the 6 sample vessel group at 574 watts for 10 minutes. This program brings the samples to 175ºC in 5.5 minutes; the temperature remains between 170-180ºC for the balance of the 10 minute irradiation period. The pressure should peak at less than 6 atm for most samples. The pressure may exceed these limits in the case of high concentrations of carbonate or organic compounds. In these cases, the pressure will be limited by the relief pressure of the vessel to 7.5 ±0.7 atm.
- Allow the vessels to cool for a minimum of five minutes before removing them from the microwave unit, with exhaust fan on. Allow the vessels to cool to room temperature before opening. The vessels must be carefully vented and uncapped in a fume hood.
- Weigh each vessel assembly. If the weight of acid plus the sample has decreased by more than 10% from the original weight, discard the digests. Determine the reason for the loss. Losses typically are attributed to use of digestion time longer than ten minutes, using too large of a sample, or having improper heating conditions. Once the source of the losses has been corrected, prepare a new set of samples for digestion.
- Sample Filtration: Shake the sample well to mix in any condensate within the digestion vessel before being opened. Filter the digestion vessel into a 50 mL

glass volumetric flask through ultra-clean filter paper. Rinse the sample digestion vessel, cap, connecting tube, and (if venting occurred) the overflow vessel into the 50 mL glass flask. Dilute to 50 mL. The samples are now ready for analysis. Concentrations are reported as "total."

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Note: Commercial kitchen or home-use microwave ovens shall not be used for the digestion of samples. The oven cavity must be corrosion resistant and well ventilated. All electronics must be protected against corrosion for safe operation.

- **Microwave oven** with programmable power settings up to atleast 600 Watts.
- The system must use PFA Teflon(R) digestion vessels (120 mL capacity) capable of withstanding pressures of up to 110 ±10 psi (7.5 ± 0.7 atm). These vessels are capable of controlled pressure relief at pressures exceeding 110 psi.
- **A rotating turntable** must be used to ensure homogeneous distribution of microwave radiation within the oven. The speed of the turntable must be a minimum of 3 rpm.
- **Polymeric volumetric labware** in plastic (Teflon(R) or polyethylene) 50 mL or 100 mL capacity.
- **Whatman No. 41 filter paper** (or equivalent).
- **Disposable polypropylene filter funnel**.
- **Analytical balance**: 300 g capacity, and minimum ± 0.01 g.
- **Polyethylene bottles**: 125 mL, with caps.
- **ASTM Type I water** (ASTM D1193): water must be monitored.
- **Sub-boiled, concentrated nitric acid** (sp. gr. 1.41).
- **Concentrated hydrochloric acid** (sp. gr. 1.19).

8.3.1.3 Total Digestion of Sediment with Hydrofluoric Acid for Trace Metal Analyses

A method is described herein for the preparation of bottom sediment samples for trace metal analysis by atomic absorption spectrophotometry (AAS). Before samples can be analyzed by AAS methods in use in this laboratory, they must be converted from solid to liquid form.

- Wet sediment is homogenized in its container, and an aliquot is freeze dried and homogenized to a fine powder.
- Approximately 0.20 to 0.25 g of powdered sediment is weighed into a Teflon reaction vessel, 3 mL of concentrated HNO_3 are added and the lid is tightened.
- The vessel is heated in a 130°C oven for 24 hrs.
- Concentrated hydrofluoric acid (2 mL) is added, the lid tightened, and the reaction vessel is heated again for 24 hrs. at 130ºC.
- Samples are then diluted to a final volume of 20 mL with 5% boric acid and transferred to a polyethylene bottles for storage until analysis.

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- **Mortar and Pestle**: To homogenize sample.
- **Reaction Vessels**: Savillex 50 mL Teflon reaction vessels or equivalent.
- **Oven**: Heated to 130°-135°C.
- **Disposable Plastic Transfer Pipets**: 1 mL.
- **Balance**: Top loading with accuracy of 0.01 g.
- **Analytical Balance**: With an accuracy of 0.0001 g.
- **Screw Top Bottles**: Nalgene or equivalent.
- **Reagent Water**: Reagent water contains no analytes above the method detection limit. Reagent water is produced by subboiling redistillation of water in a quartz still.
- **Concentrated Nitric Acid**: Baker Ultrex Grade or equivalent, stored in Teflon bottle.
- **Concentrated Hydrofluoric Acid**: Baker analyzed or equivalent.
- **Boric Acid**: Ultrex grade or equivalent.

8.3.2 Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS)

Graphite furnace atomic absorption spectrophotometry (GFAAS) relies on the electrical resistance heating generated by passing an electrical current through a graphite tube to evaporate the solvent (water), remove interfering species, and finally atomize the analyte into the light path of an absorption spectrophotometer. This technique is approximately two orders of magnitude more sensitive than Flame AAS (FAAS), primarily because the whole sample that is injected into the graphite tube is analyzed (as opposed to 10% in the case of FAAS), and because the absence of a flame results in a better signal-to-noise ratio. However, the GFAAS technique is much slower, requiring up to several minutes for individual analyses and is generally much more susceptible to interferences. GFAAS is recommended for cadmium (Cd) and lead (Pb) determinations.

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- **GFAAS**: One example of a GFAAS instrument is a Perkin Elmer Zeeman 3030 equipped with a HGA 600 furnace capable of an almost infinite range of temperatures, heating rates and holding times.
- **Autosampler**: The GFAAS is equipped with an autosampler having 40 sample positions and allows for delivery of sample and matrix modifier volumes from 1-99 µL.
- **Hollow cathode lamps**: The use of special light sources and careful wavelength selection permits the specific determination of one element in the presence of others. Hollow cathode lamps consist of a sealed glass cylinder filled with an inert gas, an anode, a cathode, and an end window. The cathode is a hollow cylinder of the element whose spectrum is to be produced.
- **Electrodeless discharge lamps**: Electrodeless discharge lamps (EDL) are typically more intense and usually provide better sensitivity than hollow cathode lamps. EDLs consist of the element of interest or a salt sealed in a quartz bulb containing an inert atmosphere. The bulb is contained in a ceramic holder on which an radio frequency coil is wound.
- **Balance**: Top loading with accuracy of 0.01 g.

- **Autosampler cups**: 2.0 mL polystyrene autosampler cups.
- **Microliter pipettes**: 1000-, 500-, 300-, 200-, 100-, 50-, 25- and 10-µL capacity.
- **Reagent water**: Reagent water contains no analytes above the method detection limit. Reagent water is produced by redistilling water in a quartz still.
- **Nitric acid**: Baker Ultrex Grade or equivalent, stored in Teflon bottle.
- **Calibration standards**: The calibration standards are prepared daily from commercially available reference standards, diluted with reagent water, and matrix-matched to the acid content of the samples. The calibration blank consists of reagent water, matrix-matched to the acid content of the samples. The highest standard's concentration is chosen to be at the upper end of either the expected sample levels or the element's linear detection range, whichever is less. Each element has appropriate calibration standard concentrations and standard operating conditions.
- **Matrix recovery spiking solution**: The volume and concentration of matrix spiking solution are element and sample dependent. In all cases, the goal is to increase the observed concentration by 50%, while not diluting the sample matrix by more than 25%. When matrix spiking results in a concentration outside the calibration range, the sample is diluted and reanalyzed.
- **Matrix modifiers**: The technique of GFAAS has been greatly advanced by the use of several matrix modifiers which remove or greatly diminish interferences from sample matrix. The modifiers assist in the analysis by either increasing the volatility of an interferent, or by increasing the analyte's thermal stability so that the sample may be charred at a higher temperature. Modifiers used for this purpose are described below.

Instrumental operating conditions, matrix modifiers, and expected sensitivities are as follows:

Element	Source	Wavelength	Slit	Site	Matrix Modifier	Char. Mass
Ag	HCL	328.1	0.7	P	0.015 mg Pd + 0.01 mg $Mg(NO_3)_2$	1.5
As	EDL	193.7	0.7	P	0.015 mg Pd + 0.01 mg $Mg(NO_3)_2$	15.
		197	0.7	P	0.015 mg Pd + 0.01 mg $Mg(NO_3)_2$	30.
Cd	EDL	228.8	0.7	P	0.2 mg PO_4 + 0.01 mg $Mg(NO_3)_2$	0.35
Cr	HCL	357.9	0.7	P	0.05 mg $Mg(NO_3)_2$	3.3
Cu	HCL	324.7	0.7	P	0.015 mg Pd + 0.01 mg $Mg(NO_3)_2$	8.0
Fe	HCL	248.3	0.2	P	0.05 mg $Mg(NO_3)_2$	5.0
Mn	HCL	279.5	0.2	P	0.05 mg $Mg(NO_3)_2$	2.2
Mo	HCL	313.3	0.7	W		9.0
Ni	HCL	232.0	0.2	P		13.0
Pb	EDL	283.3	0.7	P	0.2 mg PO_4 + 0.01 mg $Mg(NO_3)_2$	12.0
Sb	EDL	217.6	0.7	P	0.015 mg Pd + 0.01 mg $Mg(NO_3)_2$	22.0
Se	EDL	196.0	0.7	P	0.015 mg Pd + 0.01 mg $Mg(NO_3)_2$	28.0

(Cont.)

Element	Source	Wavelength	Slit	Site	Matrix Modifier	Char. Mass
Sn	EDL	286.3	0.7	P	0.015 mg Pd + 0.01 mg $Mg(NO_3)_2$	24.0
V	HCL	318.4	0.7	W	0.05 mg $Mg(NO_3)_2$	40.0
Zn	HCL	213.9	0.7	P	0.006 mg $Mg(NO_3)_2$	0.45

where HCL = hollow cathode lamp,
EDL = electrodeless discharge lamp,
Wavelength and slit width are in nm,
Site, "P" and "W" refer to "platform" and "wall" as the site of sample deposition, respectively,
Characteristic mass is the mass of the analyte, in picograms, expected to result in a peak area of 0.0044 Abs-sec.

Calibration Standards for Graphite Furnace Concentration (ng/mL)

Element	Calib. Blk.	Low Std.	Mid Std.	High Std.
Ag	0	1.5	3.0	4.5
As	0	20	40	60
Cd	0	0.4	0.8	1.2
Cr	0	4.0	8.0	12.0
Cu	0	10	20	30
Fe	0	5	10	15
Mn	0	2.5	5.0	7.5
Mo	0	10	20	30
Ni	0	15	30	45
Pb	0	15	30	45
Sb	0	45	90	135
Se	0	35	70	105
Sn	0	25	50	75
V	0	45	90	135
Zn	0	0.1	0.2	0.3

An example analytical sequence:

Sequence Number	Autosampler Position	Contents
1	1	Cal. Blk.
2	2	Low Std.
3	3	Mid Std.
4	4	High Std.
5	5	Calibration Verification Spl. (CV)
6	6	Sample
7	7	Matrix Spike (MS) into Sample 6
8	8	Sample
9	9	Sample
10	10	Sample
11	11	Sample
12	12	Sample
13	13	Sample
14	14	Sample
15	15	Sample
16	16	Sample
17	1	Cal. Blk.

(Cont.)

Sequence Number	Autosampler Position	Contents
18	2	Low Std.
19	3	Mid Std.
20	4	High Std.
21	17	Sample
22	18	Sample
23	19	Sample
24	20	Sample
25	21	Sample
26	22	Sample
27	23	Sample
28	24	Sample
29	25	Sample
30	26	Sample
31	27	Sample
32	28	Sample
33	1	Cal. Blk.
34	2	Low Std.
35	3	Mid Std.
36	4	High Std.
37	29	CV
38	30	Sample
39	31	MS into sample in pos'n 30
40	32	Sample
41	33	Sample
42	34	Sample
43	35	Sample
44	36	Sample
45	37	Sample
46	38	Sample
47	39	Sample
48	40	Dup. anal of spl from pos'n 6
49	1	Cal. Blk.
50	2	Low Std.
51	3	Mid Std.
52	4	High Std.

Matrix spikes in this listing refer to post-digestion spikes.

Sample concentrations are determined from the calibration results and from the dilution factors involved in instrumental analysis and sample digestion according to the following equation:

$$\text{Concentration}\,(\mu g/g) = [\text{Conc}_{spl} \times \text{DF}_{spl} - \text{Conc}_{PB}] \times \text{DF}_{dg'n} \div 1000$$

where:

Conc_{spl} = the concentration observed in the sample, in µg/g,
Conc_{PB} = the concentration observed in the procedural blanks, in µg/g,
DF_{spl} = the dilution factor required to dilute the sample concentration to the working range of the machine,
$\text{DF}_{dig'n}$ = the dilution factor resulting from sample digestion, in mL/g.

Reporting units are µg/g dry or weight depending on protocol requirements. The minimum method performance standard is dependent on the dilution factor resulting from digestion of sediment or soil sample and on the sensitivity of the technique for each element. General requirements for acceptable performance include instrumental sensitivity that is no less than 80% of that reported by the manufacturer or from historical records (instrumental characteristic masses listed above). Results are reported to the number of significant figures that matches the number of such figures in the absorption reading. Samples with absorption less than 0.010 are reported with only 1 significant figure, samples with absorption equal to or greater than 0.010 and less than 0.100 have two significant figures, and samples with absorption greater than or equal to 0.100 are reported with three significant figures.

Quality control samples are processed in a manner identical to actual samples. One method blank is analyzed with every 20 samples or with every sample set, whichever is more frequent. Blank levels should be no more than twice the method detection limit (MDL). If blank levels are > 2x MDL, the analysis is repeated and the data reported if QC criteria are met. If blank results are still > 2x MDL, the sample set is reprocessed after the source of contamination is isolated. If insufficient sample is available, data are reported with a blank correction and noted as such in the data.

One reference material is analyzed with every 20 samples or with every sample set. Sediment or water certified reference materials, if available, and as closely matching the sample set as possible, are analyzed with each sample set. Reference materials include:

Sediment samples:
- BCSS-1 (NRCC, Canada),
- MESS-2 (NRCC, Canada), and
- Estuarine sediment, #1646 (NIST, U.S.),.

Water samples:
- CASS-2 (NRCC, Canada), and
- SLRS-1 (NRCC, Canada).

Reference material is deemed acceptable if the results are within 20% of the certified value (if this value is ≥ 2x MDL) or within the 95% confidence interval, whichever is greater.

One matrix spike is analyzed with every 20 samples or with every sample set. Matrix spikes are used to investigate possible interferences which may result in either signal enhancement or suppression. Spiked and unspiked sample concentrations are compared and spike recovery is calculated according to the equation:

$$\text{Spike Recovery } (\%) = \frac{C_{spkd\,spl} - C_{spl}}{C_{spk} \times \frac{V_{spk}}{V_{spkd\,spl}}} \times 100$$

where:

$C_{spkd\ spl}$ = the concentration observed in the spiked sample,
C_{spl} = the concentration observed in the unspiked sample,
C_{spk} = the concentration of the spiking solution,
V_{spk} = the volume of the spike solution used in making the spiked sample,
$V_{spkd\ spl}$ = the volume of the spiked sample = $V_{spl} + V_{spk}$

Spikes are deemed valid only if the analyte concentration of the sample is < 2x MDL and the spiked concentration is doubled or increased by ≥ 4x the MDL, whichever is greater. Only valid spikes can be judged acceptable or unacceptable. When spike concentration is less than 10% of the sample concentration, calculations for matrix spikes are not performed.

One post-digestion matrix spike is analyzed with every 20 samples or with every sample set. These spikes are used to investigate possible interferences which may result in either signal enhancement or suppression. A small volume of a standard is added to a portion of the sample, which is then analyzed. Spiked and unspiked sample concentrations are compared and spike recovery is calculated according to the equation:

$$\text{Spike Recovery (\%)} = \frac{C_{spkd\,spl}\,V_{spkd\,spl} - C_{spl}\,V_{spl}}{C_{spk}\,V_{spk}} \text{x}100$$

where:

$C_{spkd\ spl}$ = the concentration observed in the spiked sample,
$V_{spkd\ spl}$ = the volume of the spiked sample = $V_{spl} + V_{spk}$,
C_{spl} = the concentration observed in the unspiked sample,
V_{spl} = the volume of sample used in making the spiked sample,
C_{spk} = the concentration of the spiking solution, and
V_{spk} = the volume of spike solution used in making the spiked sample.

Spikes are deemed valid only if the analyte concentration is < 2x MDL and the spiked sample concentration is doubled or increased by ≥ 4x the MDL, whichever is greater. Only valid spikes can be judged acceptable or unacceptable.

One duplicate sample is analyzed with every 20 samples or with every sample set. Inhomogeneous samples may result in greater variability between duplicates. Experience has indicated that reference materials are more homogeneous than are samples, and thus comparison of the RPD of duplicates for a) reference material duplicate analyses, b) sample duplicate analyses and c) duplicate analyses from single digestion solutions gives an indication of a) total analytical variability (i.e., processing + instrumental variability), b) the sum of analytical variability and natural sample inhomogeneity, and c) instrumental variability. For standard QA purposes, “duplicate analysis” consists of preparation and analysis of duplicate sample aliquots. An RPD of ± 20% is deemed acceptable for valid sample duplicate analyses (i.e., having analyte concentrations ≥5x MDL or other values as required).

Two blank spikes are analyzed with every 40 samples or with every sample set. Blank spikes are method blanks that have been fortified and carried through the digestion and analytical procedures. Blank spikes are analyzed to identify any digestion interferences or losses, and to evaluate the performance of the analysis. Blank spike recovery is acceptable when it is within 80-120% of the known value. If the recovery is outside these limits, the analysis is repeated and the re-analysis data is reported if it meets the criterion. If more than 10% of the blank spikes in a delivery set are deemed unacceptable then samples corresponding to these analytical batches are redigested.

8.3.3 Inductively Coupled Plasma Emission Spectroscopy (ICP)

The following procedures are used for the analysis of trace metals using Inductively Coupled Plasma (ICP) Emission Spectroscopy. The ICP techniques use a Leeman Labs PS-3000 UV Inductively Coupled Plasma Spectrometer with Echelle grating optics and a sequential/simultaneous combination detection system or equivalent instrumentation. This method is recommended for copper (Cu) and zinc (Zn) determinations.

These instrumental procedures can be used for the analysis of trace metals in digestates resulting from preparation of water, snow and sediment samples. The routine trace metals quantitatively determined by this method, with their suggested wavelengths, are provided in Table 8.1. The following analytes can be determined semi-quantitatively using the procedures described in EPA Method 1620: Bi, Ce, Dy, Er, Eu, Gd, Ga, Ho, In, La, Lu, Nd, P, Sm, Sc, Sr, Tb, Tm, U, Yb, Ge, Au, Hf, Ir, Li, Nb, Pd, Pt, K, Rh, Re, Ru, Si, Ta, Te, Th, Zr, W, Os, Pr, and S.

Because it is difficult to find a “clean” matrix free of trace metals, instrument detection limits (IDLs) are routinely used for ICP analyses instead of method detection limits. The IDL is established by performing seven consecutive measurements using a standard solution at a concentration three to five times the estimated IDL. These measurements are performed on three non-consecutive days and the resulting average standard deviation is multiplied by three to determine the IDL. If multiple instruments are used, an IDL is determined for each instrument. The IDL is determined quarterly and the highest IDL is used during that quarter for reporting all data from all ICPs. The IDL represents the minimum concentration of an element that can be measured and reported with 99% confidence that the element concentration is greater than zero.

The method detection limit (MDL) is defined as the minimum concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. MDLs are determined by the preparation, digestion, and analysis of a “clean” sample matrix, either spiked with or containing the target analytes, following the procedures required by 40 CFR, Part 160, Appendix B. The composition of the routine analytical standards used for instrument calibration are provided in Tables 8.2 and 8.3. Calibrations are linear over the range of the standards used. Quarterly linear range studies prove that the linearity of the instrumental determinations extends beyond the highest calibration standards for most elements. When sample concentrations exceed the linear range, the sample is diluted and re-analyzed. There are no significant spectral overlaps found for the wavelengths listed in Table 8.1. An inter-element correction factor study is performed annually. As a result, no significant interelement correction factors have been found to be necessary. Background correction points are used to compensate

Table 8.1. Wavelength Used for Routine ICP Quantitative Trace Metal Analytes.

Element		Wavelength (nm)
Aluminum	Al	308.215
Antimony	Sb	206.833
Arsenic	As	193.695
Barium	Ba	455.403
Beryllium	Be	234.861
Boron	B	249.678
Cadmium	Cd	214.438
Calcium	Ca	317.933
Chromium	Cr	267.716
Cobalt	Co	228.616
Copper	Cu	324.754
Iron	Fe	259.940
Lead	Pb	220.353
Magnesium	Mg	279.079
Manganese	Mn	257.610
Molybdenum	Mo	202.030
Nickel	Ni	231.604
Potassium	K	766.490
Selenium	Se	196.026
Silver	Ag	328.068
Sodium	Na	589.592
Strontium	Sr	421.552
Tin	Sn	189.946
Titanium	Ti	334.941
Vanadium	V	310.230
Yttrium	Y	371.030
Zinc	Zn	206.200

for matrix related spectral interferences. The analyst has the responsibility for applying background correction depending on the particular wavelength, matrix, concentration range and calibration equation applied. Background correction is required for the weighted linear calibration used by this ICP's software. Physical interferences associated with sample nebulization and transport processes may be observed when samples with high amounts of total dissolved material are analyzed. The use of a peristaltic pump significantly lessens these interferences. However, samples with high dissolved solids may require dilution prior to analysis. If the estimated amount of total dissolved material in a diluted sample is higher than 1000 ppm, the matrix matching techniques should be used. Method specific quality control (QC) acceptance criteria are summarized in Table 8.4.

A peaking routine should be performed after the instrument has warmed up at least 30 minutes and prior to sample analysis. "Peaking" on the X and Y axes locates the best viewing position in the plasma and checks nebulizer argon pressure. Peaking should be performed using a manganese solution of 20-100 ppm. Peaks should be centered and even. If the Y peak is uneven or erratic, the nebulizer may need to be adjusted or a leak may have been detected.

Table 8.2. Calibration Standard Concentration.

Standard Identifier	Analytes and their concentrations (µg/mL)		Matrix
(Also used as CCB)	Ca, Na, K	200	10% Nitric Acid
S4* ScanM	Al, Ca, Fe, Mg, Na, K	100	5% Nitric Acid
	Ba, Sb	10	
	Be, Cd, Co, Cu, Mn	5	
	Ni, V, Zn	5	
S3* ScanM (Also used as ICV)	Al, Ca, Fe, Mg, Na, K	20	5% Nitric Acid
	Ba, Sb	1	
	Be, Cd, Co, Cu, Mn	0.5	
	Ni, V, Zn	0.5	
S2* ScanM	Al, Ca, Fe, Mg, Na, K	4	5% Nitric Acid
	Ba, Sb	0.1	
	Be, Cd, Co, Cu, Mn	0.05	
	Ni, V, Zn	0.05	
S1* ScanM/Q (Also used as CCB)			5% Nitric Acid
S4* ScanQ	As, Pb, Se	10	5% Nitric Acid
	B, Mo, Sr, Cr	5	
S3* ScanQ (Also used as ICV)	As, Pb, Se	1	5% Nitric Acid
	B, Mo, Sr, Cr	0.5	
S2* ScanQ	As, Pb, Se	0.1	5% Nitric Acid
	B, Mo, Sr, Cr	0.05	

*Standards are used for sediment calibration standards.

Consult the ICP manual to correct the problem. Prior to sample analysis, a daily instrument QC test is performed to ensure that the instrument, particularly the sample introduction components, are operating correctly. Performance is determined by evaluating the reproducibility of a daily QC solution which has been analyzed twenty consecutive times. The relative standard deviation (RSD) calculated by the instrument software for each analyte should be $\leq$1%. If the RSD exceeds the QC acceptance criterion, corrective action is taken. The daily QC data is stored in the file for daily QC runs.

A 4-point calibration curve, using background correction, is used in this procedure. The ICP is linear over a wide concentration range. The ICP software uses a weighted linear least-square fit for low-level determinations which permits low concentrations to have more of an impact on the fit of calibration line. The correlation coefficient should be $\geq$0.9950 for each analyte in the calibration curve. The absolute value of the concentration for an element in the calibration blank should be less than the ML for each element in question. The RSD for three replicates at 10 ppm level should be $\leq$1% and at 1 ppm the RSD should be $\leq$5% for most elements. Significantly worse precision may be due to inadequate uptake time, exhausting the standard, sample introduction problems, solution chemistry, or wavelength alignment. The initial calibration is stored in the appropriate ICP calibration file.

A matrix matched blank (CCB; Table 8.2) is analyzed after each calibration and/or at the beginning of each analytical set. If the absolute calculated value for any analyte is higher than the ML, corrective action is taken. Corrective action may include a calibration update, instrumental maintenance and/or recalibration. A matrix matched blank (CCB; Table 8.2) is analyzed after each 10 samples or after each 2 hour period (whichever is sooner) and at the

completion of each analytical set. If the absolute calculated value for any analyte is higher than the ML, corrective action is taken. Corrective action may include terminating analysis, a calibration update, instrument maintenance and/or recalibration. A matrix matched low (ICV, Table 8.2) or mid-level standard (CCV, Table 8.3) is analyzed after calibration and/or at the beginning of each set. The QC acceptance criteria required for each analyte is a concentration determination of 90 to 110% of the true value. If any analyte value is outside of the acceptance criteria, corrective action is taken, which may include a calibration update, instrument maintenance and/or recalibration. A matrix matched mid-level standard (CCV, Table 8.3) is analyzed after each 10 samples or after each 2 hour period (whichever is sooner) and at the completion of each analytical set. The QC acceptance criteria required for each analyte is a concentration determination of 90 to 110% of the true value. If any analyte value is outside of the QC acceptance criteria, corrective action is taken, which may include terminating analysis, a calibration update, instrumental maintenance and/or recalibration.

As a result of changes in the laboratory environment, the light intensities measured for a given concentration may change slightly. A corrective "calibration update" procedure can be used to adjust the calibration line. There are two update options, the intercept update and the slope update, which are described in the following sections. The intercept update should always be performed first, especially if problems are indicated with the calibration blank (ICB/CCB) standard. The slope update follows the intercept update, if needed, when there are problems with the low or mid-level check standard (CK2, ICV or CCV). The "Intercept update" determines an additive correction factor and is calculated after analyzing a single intercept update standard (standard 1; S1; Table 8.2) in this procedure. The QC acceptance criteria requires that the intercept correction for each analyte should be $\leq$2 times the CRDL or ML or recalibration should be performed. The "slope update" determines a multiplicative correction factor to the calibration equation and is calculated after the intercept update by analyzing a single slope update standard containing all elements (standard 4; S4; Table 8.2) in this procedure. The QC acceptance criteria requires that the % slope correction for each analyte included in the slope update should be $\leq$10% or recalibration procedures should be performed. The continuing calibration blank (CCB) and continuing calibration verification (CCV) standards are used to validate the calibration update(s).

All data from QC samples are evaluated for a specific digestion batch and analytical sequence before making decisions regarding corrective actions. However, contamination of the method blank normally requires complete analysis of the batch, unless the analyte concentration in all samples is 10 times that in the blank. A method blank is used to demonstrate that sample preparation, digestion, and analytical procedures are free of contamination. A method blank is required for each set of 20 or fewer samples in a preparation/digestion batch. The QC acceptance criteria for the method blank specifies that if the target analytes are present in concentrations >2 times the ML, the preparation/digestion batch requires re-digestion, unless the concentration in a sample is 10 times that found in the method blank. Analyst discretion is used when contamination is present which does not adversely affect the overall analysis.

A sample duplicate (DUP) is used to evaluate matrix homogeneity and analytical precision in the presence of a representative matrix and is required with each set of 20 or fewer samples. The QC acceptance criteria for duplicate analyses is that the relative percent difference (RPD) between the original and its duplicate should not exceed 20%. The DUP QC acceptance

Table 8.3. Check 2 Standard Concentration.

Standard Identifier	Analytes and their concentrations (µg/mL)		Matrix
CCV Tissim, Tisseq	Ca, K, Na	20	10% Nitric Acid
	Al, Fe, Mg	5	
	Ba, Be, B, Cr, Cu	0.5	
	Mn, Mo, Ni, Sr, V, Zn	0.5	
CCV ScanM/Q	Al, Ca, Fe, Mg, Na	52	10% Nitric Acid
	K	20	
	Ba, Be, B, Sb, As, Cd	2	
	Cr, Co, Cu, Fe, Pb, Li	2	
	Mg, Mn, Mo, Ni, Se, Sr	2	
	Tl, Ti, V, Zn	2	
	Ag	1	

Table 8.4. Key Elements of ICP Quality Control.

Element	Control Limit Criteria	Frequency
1. Initial and Periodic Analytical Requirements		
- Instrument Detection Limit (IDL), All Instruments	Must meet Minimum Levels (MLs) specified in contract or EPA Method 1620.	Within 30 days of start of contract analysis and quarterly during contract period or after major instrument adjustment.
- Initial Precision and Accuracy, All Instruments	Recovery range of 75-125% for each element; %RSD meets EPA Method 1620, Table 8 criteria.	Initial for each type of instrumental analysis, annually thereafter.
- Analysis of Performance Evaluation (PE) Samples	Minimum passing score is 75 out of 100 points.	Initial and at regular intervals throughout the year
- ICP Interelement Correction Factors	Report any correction factors.	Prior to beginning contract analysis, annually thereafter and after major instrument adjustment.
- ICP Linear Range Verification Check Standard Analysis (LRV)	Within ± 5% of true value for quantitative ICP analytes.	For each element at each wavelength and then quarterly during contract period.
2. QC Requirements for ICP Quantitative Analysis		
- Instrument Calibration	Minimum of a blank and one standard; correlation coefficient of 0.995 or better. Baseline correction or resloping acceptable if preceded and followed by ICV/ICB or CCV/CCB.	Each time the instrument is set-up (initialized) and then each 24 hours during a continuous run.
- Initial Calibration Verification (ICV)	Within ±10% of the true value. If fails, stop, recalibrate, and reanalyze.	Immediately after system calibration and at beginning of each analysis run.

Table 8.4. (Cont.)

Element	Control Limit Criteria	Frequency
- Initial Calibration Blank (ICB)	Absolute value □ML for quantitative analyses. When fails, recalibrate, reprocess with ICV, ICB.	After every ICV.
- ICP Minimum Level Standard Solution	Use ICP standard at 2x ML or 2x IDL, whichever is greater. Compare to MLs on Table 3.2; control limits not yet specified; report recoveries.	After ICV/ICB and at end of each analysis run or twice per 8-hour shift, whichever is more frequent. CCV, CCB must follow analysis of the sample.
- Continuing Calibration Verification (CCV)	Within ±10% of the true value. If fails, stop, recalibrate, and reanalyze back to last passing CV. Same CCV must be used for entire Episode or sample set.	At a minimum of every 10% or every 2 hours during an analysis run, and after the last analytical or QC sample.
- Continuing Calibration Blank (CCB)	Absolute value □ML for quantitative analyses. When fails, recalibrate and reanalyze back to last acceptable CB.	After every CCV.
- Interference Check Samples for ICP	The IC must be within ± 20% of the true value of the solution after consecutive runs of IC. If fails, stop, correct, recalibrate, and reanalyze back to last acceptable ICS pair.	Analyze consecutively at beginning and end of each analysis run or twice each 8 hours shift.
- Laboratory Control Sample (LCS)	Recovery within 80 - 100% (except for Ag), or if fails, terminate analysis; redigest and reanalyze. For Ag, qualify results outside 80-120% and report.	One LCS per sample set or Episode, whichever is more frequent.[2]
- Preparation Blank (PB)	Absolute value ≤ the ML; if it fails, the associated samples > ML and ≤ 10 ML must be reprocessed; samples > 10 ML can be reported.	One PB with each sample set prepared.
- ICP Digested Matrix Spike[3]	Recovery range 75% to 125%; repeat analysis if fails; then recalibrate if fails; if this continues to fail, dilute by 10, report and qualify.	At least 10% of samples analyzed per matrix per sample set.
- ICP Digested Matrix Spike Duplicate[4]	Same recovery criteria as for matrix spike. RPD □20% is acceptable range for inorganic elements; report and qualify failure.	At least 10% of samples analyzed per matrix per sample set.
- ICP Serial Dilution Analysis	If analyte is 50x IDL, the % difference must agree within 10% of original, or flag all associated data if > 10%.	On 10% of samples analyzed or at least one per sample set whichever is more frequent.

Note 1: Analysis of Standard Reference Materials (SRM) as an LCS requires a recovery ± 20% of certified values for complete digestions only.

Note 2: A sample set is a group of up to 20 field samples (20 samples or less) prepared at the same time and associated with the same QC samples.

Note 3: Samples to be spiked are normally specified by the client; matrix spike solutions contain selected analytes of interest. The spike concentration of each analyte in the solutions 1-5 x background sample level or 5-50 x MDL for non-detects.

Note 4: RPD = Relative percent difference between spike recovery results for matrix spike and matrix spike duplicate.

criteria are advisory only and exceedance may not require re-digestion of the entire analytical batch. However, if more than two analytes are outside the QC acceptance criteria, corrective action is indicated. Corrective action may include re-analysis of the DUP and the original sample, instrument maintenance and/or recalibration, or re-digestion of the original sample and its duplicate. The RPD is considered invalid and is not evaluated when results are less than 10 times the ML.

A matrix spike (MS) sample is used to evaluate analytical accuracy in the presence of a representative matrix, and is required with each set of 20 or fewer samples. A matrix spike duplicate (MSD) is used to evaluate both analytical accuracy and precision in the presence of a representative matrix, and is required with each set of 20 or fewer samples. The QC acceptance criteria for analytes in the MS and MSD is a recovery of 75 to 125% of the spiked amount. The QC acceptance criteria is invalid when the spike amount is not sufficient to increase the analyte concentrations in the sample by at least 50%. If a MSD has been included with the analytical batch, the results obtained from the MS and MSD samples should agree within a RPD of 20%. The MS and MSD QC acceptance criteria are advisory only and exceedance does not require re-digestion of the entire analytical batch. However, if more than two analytes are outside the acceptance criteria, corrective action is indicated. Corrective action may include reanalysis of the original sample and its MS/MSD, instrument maintenance and/or recalibration or re-digestion of the original sample, and its MS/MSD.

A standard reference material (SRM) is used to evaluate analytical accuracy for a certified reference matrix from an independent source, and is required with each set of 20 or fewer samples. The QC acceptance criterion for measured analyte concentrations in SRM is 75 to 125% recovery of the laboratory average or the certified concentration. The QC acceptance criteria is invalid when the certified analyte concentration falls below the ML. The SRM acceptance criteria are advisory only and exceeding the QC acceptance criteria does not require re-digestion of the entire analytical batch. However, if more than two analytes are outside the acceptance criteria, corrective action is indicated. Corrective action may include reanalysis of the SRM, instrument maintenance and/or recalibration or re-digestion.

In the absence of an SRM, a laboratory blank spike (LBS) may be used to evaluate analytical accuracy of the method, and may be required with each set of 20 or fewer samples. The QC acceptance criterion for the LBS analyte recoveries is 80 to 120% of spiked amount. If more than two analytes are outside the acceptance criterion, corrective action is indicated. After evaluation of other QC samples, corrective action may include re-analysis of the LBS, instrument maintenance and/or recalibration, or re-digestion of the entire batch.

When adequate digestate is available, an instrumental spike (IS) may be used to demonstrate matrix effects on the sample or on the introduction system, and may be required with each digestion set. A calibration standard spike analysis can be used to replace the IS if inadequate digestate is available. The QC acceptance criterion for analyte recoveries in the IS is 90 to 110% of the spiked amount. The IS acceptance criteria are advisory only. However, if more than two analytes are outside the QC acceptance criteria, corrective action may be indicated. Corrective action may include dilution, use of serial dilutions, or redigestion of the entire analytical batch.

The Leeman Labs PS3000 UV instrument uses a hot argon plasma to serve as an excitation source. This is produced by a 40.68 MHz free-running oscillator that can operate at any ICP power level up to 2.0 kW. The quartz ICP torch has three concentric tubes, each carrying a flow of argon. The outer tube carries the "coolant" flow, which acts as a barrier between the quartz and the extremely hot plasma. The coolant also provides the argon for the plasma itself. The central tube carries the "auxiliary" flow, and the inner injector tube carries the sample aerosol in the "nebulizer" flow. Four operating parameters are used to control the hot argon plasma's condition: generator power, coolant argon flow, auxiliary argon flow, and nebulizer pressure. A fifth, the delivery rate of the peristaltic pump, is also critical to analytical performance. All these operating parameters vary according to the analytical procedure. Specific criteria should be stored under each analytical method name in the ICP software. Hard copies of the analytical method screens should be maintained in the trace metals laboratory office file. Typical operating parameters for the Modified Scott Spray Chamber used for this operating procedure are shown in Table 8.5. These parameters will be different for other instruments. These are provided as guidance.

Table 8.5. Typical Analytical Protocol Operating Parameters for the Modified Scott Spray Chamber.

Parameters	Aqueous	High Solids	Alkali	Organic
Power, kW	1.0	1.0-1.2	0.7	1.2
Coolant, L/min	10-13	11-16	12	11-16
Auxiliary, L/min	0-0.5	0-1.0	0	0.4-1.0
Nebulizer, p.s.i.	35-46	42-47	35-60	30-38
Pump Rate, mL/min	1.0-1.6	1.0-1.6	0.9-1.0	0.9-1.1
Peaking Element	Mn	Mn	Ca, Na, Li, Al	Fe, Ni

Before ignition: Check pump tubing and replace if worn.
Verify and if necessary adjust pump tension.
Verify and if necessary adjust torch alignment.
Check nebulizer operation before turning on the plasma.
Check the interlock panel to make sure that there are no lights on.

After ignition: Warm up the instrument for at least 45 minutes before performing analysis.

To establish the best optical position (horizontal and vertical), a software peak optics procedure called "Peak Source" should be performed each time the ICP is started and after any change in operating conditions. To maintain optical stability, the instrument should be programmed to automatically perform peak source procedure every 20 minutes or after 5 samples, whichever is greater. Daily QC procedures must be performed after proper warm up time to ensure that the instrument, particularly the sample introduction components, are operating correctly.

Analytical standards are purchased or prepared as solutions. Standards are stored in plastic containers (HDPE or LDPE Nalgene) at ambient temperature. Purchased standards are certified to ensure their purity, concentration, and authenticity. Prepared standards must meet the

following QC acceptance criteria. All target analytes should be present at ± 5% of the expected concentrations. For calibration verification, analysis of an EPA or NIST-traceable analytical standard using the new calibration standards should meet all QC acceptance criteria.

A four (4) point calibration curve is used for most trace metal analyses by ICP. Standard 4 (S4) is the high concentration solution, Standard 1 (S1) is the blank, and Standards 2 and 3 (S2 and S3) are intermediate standard concentrations. After the ICP is calibrated, the calibration curve is evaluated using the analysis of initial and continuing calibration blanks and standards. Calibration standards are either purchased as custom multi-element solutions or prepared in-house from purchased single element solutions (see Table 8.6) at the concentrations included in Table 8.3. As required, calibration standards are diluted using the appropriate Class A volumetrics and/or calibrated pipettors with a 5% or 10% nitric acid solution, depending on the analytical procedure being used.

Spiking solutions are prepared for each matrix. Standards are prepared in 5% nitric acid using the appropriate Class A volumetric glassware and/or calibrated pipettors. Samples (and LBS when required) are spiked with the appropriate spiking solution at a minimum of 1 per 20 or fewer samples for each digestion batch. Matrix spike composition for sediments are listed in Table 8.8.

Using Class A volumetric glassware, a daily QC solution is prepared using single element stock solutions (see Table 8.6) for a final content of 1 ppm Ba and 10 ppm Cd, Cu, Fe, Mn, and Zn in a 10% nitric acid matrix. The Daily Instrument QC solution is analyzed once every 24 hours or whenever the instrument is set-up. Once a month, instrument linearity should be verified. The daily QC solution should be used undiluted as standard 4, and should be diluted 1:2 for standard 3 and 1:10 for standard 2. Standard 1 is a blank solution. The correlation coefficient should be $\geq$0.995 for these analyses and solution analyte concentrations determined should be ±10% of the expected concentration.

A continuing calibration verification standard (CCV) is analyzed every 10 samples or 2 hours. This sample is prepared using Quality Control Standard 3 (ICM-224L, Table 8.7) and Quality Control Standard 4 (ICM-225L, Table 8.7) diluted in 10% nitric acid to the appropriate concentration(s). Single element stock solutions may also be diluted in 10% nitric acid to the appropriate concentrations for this check standard. A continuing calibration blank standard (CCB) is analyzed every 10 samples or every 2 hours, prior to the calibration verification standard (CCV). The composition of CCB is provided in Table 8.2.

One sample (sediment or water) per digestion batch is spiked with the CCV solution at the instrument. For a tissue digestion, the CCV solution is diluted with 5% nitric acid and analyzed as a blank spike or calibration spike.

When available, SRM is analyzed with each digestion batch to evaluate both digestion procedures and instrument performance. The SRM is matrix matched to the matrix being digested.

Choose the analytical method from the ICP software according to the sample matrix type and required analyte list. See the examples of the methods in the ICP PS-series software or

Table 8.6. Single Element Stock Solutions for ICP.

Analyte	Concentration	Matrix
aluminum	10,000 µg/mL	4% HCl
aluminum	1000 µg/mL	4% HCl
antimony	1000 µg/mL	4% nitric
arsenic	1000 µg/mL	4% nitric/tr. HCl
barium	1000 µg/mL	4% nitric acid
beryllium	1000 µg/mL	4% nitric acid
bismuth	1000 µg/mL	5% nitric acid
boron	1000 µg/mL	water
cadmium	1000 µg/mL	4% nitric acid
calcium	10,000 µg/mL	4% nitric acid
calcium	1000 µg/mL	4% nitric acid
chromium	1000 µg/mL	4% HCl
cobalt	1000 µg/mL	4% nitric acid
copper	1000 µg/mL	4% nitric acid
cerium	1000 µg/mL	5% nitric acid
dysprosium	1000 µg/mL	5% nitric acid
erbium	1000 µg/mL	5% nitric acid
europium	1000 µg/mL	5% nitric acid
gadolinium	1000 µg/mL	5% nitric acid
gallium	1000 µg/mL	5% nitric acid/tr HCl
germanium	1000 µg/mL	5% nitric acid/tr HF
gold	1000 µg/mL	20% HCl
hafnium	1000 µg/mL	5% HCl
holmium	1000 µg/mL	5% nitric acid
indium	1000 µg/mL	5% nitric acid
iridium	1000 µg/mL	20% HCl
iron	1000 µg/mL	4% nitric acid
lanthanum	1000 µg/mL	5% nitric acid
lithium	1000 µg/mL	5% nitric acid
lead	1000 µg/mL	4% nitric acid
lutetium	1000 µg/mL	5% nitric acid
magnesium	10,000 µg/mL	4% nitric acid
manganese	10,000 µg/mL	4% nitric acid
manganese	1000 µg/mL	4% nitric acid
magnesium	1000 µg/mL	4% nitric acid
molybdenum	1000 µg/mL	water
neodimium	1000 µg/mL	5% nitric acid
nickel	1000 µg/mL	4% nitric acid
niobium	1000 µg/mL	2% HF
osmium	1000 µg/mL	15-20% HCl
palladium	1000 µg/mL	20% HCl
phosphorus	1000 µg/mL	5% nitric acid
platinum	1000 µg/mL	20% HCl
potassium	10,000 µg/mL	4% nitric acid
potassium	1000 µg/mL	4% nitric acid
praseodymium	1000 µg/mL	5% nitric acid
rhenium	1000 µg/mL	5% nitric acid
rhodium	1000 µg/mL	20% HCl
ruthenium	1000 µg/mL	20% HCl
samarium	1000 µg/mL	5% nitric acid
scandium	1000 µg/mL	5% nitric acid
selenium	1000 µg/mL	4% nitric acid

Table 8.6. (Cont.)

Analyte	Concentration	Matrix
silicon	1000 µg/mL	water
*silver	1000 µg/mL	4% nitric acid
sodium	10000 µg/mL	4% nitric acid
sodium	1000 µg/mL	4% nitric acid
strontium	1000 µg/mL	4% nitric acid
sulfur	1000 µg/mL	Water/5% nitric acid
tantalum	1000 µg/mL	2% HF
tellurium	1000 µg/mL	20% HCl
terbium	1000 µg/mL	5% nitric acid
thallium	1000 µg/mL	4% nitric acid
thulium	1000 µg/mL	5% nitric acid
tin	1000 µg/mL	20% HCl
tin	1000 µg/mL	20% HCl + 2% nitric acid
titanium	1000 µg/mL	5% nitric acid/tr HF
titanium	1000 µg/mL	20% HCl+2% nitric acid
tungsten	1000 µg/mL	5% nitric acid/tr HF
vanadium	1000 µg/mL	4% nitric acid
ytterbium	1000 µg/mL	5% nitric acid
yttrium	1000 µg/mL	5% nitric acid
zinc	1000 µg/mL	4% nitric acid
zirconium	1000 µg/mL	5% HCl

*in amber bottle or cover bottle with foil.

Table 8.7. Multi-Element Stock Solutions for ICP.

Description	Use
Quality Control standard 3 100 µg/mL: Sb, As, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, Se, Sr, Tl, Ti, V, Zn in 5% nitric acid	To be diluted for use in CK2 solution (for scan M/Q) or for calibrating on Quant M or Quant Q.
Quality Control standard 4, solution 1 of 2 1000 µg/mL: K 100 µg/mL: Al, Ba, B, Na 50 µg/mL: Ag in 5% nitric acid	To be diluted for use in CK2 solution (for scan M/Q) or for calibrating on Quant M or Quant Q.
Semiquantitative standard 1 10 µg/mL: Al, As, Ba, Bi, Cd, Ca, Ce, Dy, Er, Eu, Gd, Ga, Ho, In, La, Pb, Lu, Mg, Hg, Nd, P, Sm, Sc, Se, Ag, Na, Sr, Tb, Tl, Tm, U, Yb, Y in 40% aqua regia	For calibrating EPA SEMIQ1 procedure
Semiquantitative standard 2 10 µg/mL: Sb, Be, B, Cs, Cr, Co, Cu, Ge, Au, Hf, Ir, Fe, Li, Mn, Mo, Ni, Nb, Pd, Pt, K, Rh, Re, Rb, Ru, Si, Ta, Te, Th, Sn, Ti, W, V, Zn, Zr in 40% aqua regia	For calibrating EPA SEMIQ2 procedure
Primary Analytes B standard 100 µg/mL: Cd, Pb, Ni, Ag, Zn 50 µg/mL: Ba, Be, Cr, Co, Cu, Mn, V in 5% nitric acid	To be diluted for interferents checks
Primary Interferents A standard 2500 µg/mL: Al, Ca, Mg 1000 µg/mL: Fe	To be diluted for interferents check

Table 8.7. (Cont.)

Description	Use
Initial Calibration Verification standard 1 100 µg/mL: Ba, Co, Cu, Fe, Pb, Mn, Ni, Tl, Zn 50 µg/mL: Cd 40 µg/mL: Be 20 µg/mL: Ag in 5% nitric acid	To be diluted for ICV check standard
Initial Calibration Verification standard 2 1000 µg/mL: Ca, Mg, K, Na 100 µg/mL: Al, Sb, As, Cr, Se, V in 5% nitric acid	To be diluted for ICV check standard
Continuing Calibration Verification Standard 1 500 µg/mL: Ba, Co, Cu, Fe, Pb, Mn, Ni, Tl, Zn 250 µg/mL: Cd 200 µg/mL: Be 100 µg/mL: Ag in 5% nitric acid	To be diluted for CCV check standard
Continuing Calibration Verification Standard 2 (solution 1 of 2) 500 µg/mL: Al, As, Cr, Se, V in 5% nitric acid	To be diluted for CCV check standard
Continuing Calibration Verification Standard 2 (solution 2 of 2) 500 µg/mL: Sb in 5% nitric acid	To be diluted for CCV check standard
Continuing Calibration Verification Standard 3 2500 µg/mL: Ca, Mg, K, Na in 4% nitric acid	To be diluted for CCV check standard

Table 8.8. Matrix Spike Constituents for Sediments.

Analyte	Concentration µg/mL	Spike Amount
Mn	250	1mL of multielement spike solution per 1g of a sample for Mg and mixed analyte
Zn	200	1mL of multielement spike solution per 1g of a sample for Mg and mixed analyte
B, Sr	100	1mL of multielement spike solution per 1g of a sample for Mg and mixed analyte
Ba, Cu, Mo, Se, V	50	1mL of multielement spike solution per 1g of a sample for Mg and mixed analyte
Ni	20	1mL of multielement spike solution per 1g of a sample for Mg and mixed analyte
Cr	25	1mL of multielement spike solution per 1g of a sample for Mg and mixed analyte
As, Be, Pb	10	1mL of multielement spike solution per 1g of a sample for Mg and mixed analyte
Cd	5	1mL of multielement spike solution per 1g of a sample for Mg and mixed analyte

review the hard copy maintained in the trace metals laboratory office. Set appropriate rinse and uptake time (not less than 45 seconds and 30 seconds, respectively). Check the ICP calibration as described. Analyze samples and all related QC samples under the same conditions as standards (same integration time, background correction points, plasma conditions etc.). After the analysis is completed, the data is reported in appropriate tables.

The following sections provide a summary of both routine and preventive maintenance for the Leeman Lab PS-3000 ICP. Analysts using equivalent instrumentation should follow the recommended manufacturers procedures. All maintenance and repairs made to the ICP are recorded in the "ICP Maintenance Log". Perform Peak Source and Daily QC Test. Poor results will indicate need for maintenance. This may include (but is not limited to) the following:

a. Cleaning torch and/or spray chamber. This should be done only when needed.
b. Cleaning or replacing the nebulizer.
c. Checking for gas leaks and replacing connectors or tubing if necessary. When analyzing samples dissolved in an organic matrix, clean the Torchbox daily. Failure to thoroughly clean flammable organics could lead to a fire.

Change sample introduction pump tubing at least once per week. Check uptake pump tubing to see if it needs to be changed more often (every 2-3 days). Check sipper. Change as necessary. Clean and lubricate the autosampler once a week. Check air filters on power supply, spectrometer and water recirculator once a week. Clean as necessary. Check radio frequency contact strip for corrosion and looseness. Replace as necessary.

Check nebulizer and nebulizer adapter o-rings at least once every two weeks. Change O-rings at least once a month. Oil sample introduction pump. Clean air filters on power supply, spectrometer, and water recirculator at least once each month. Drain water recirculator and refill with fresh dionized water. Check other sample introduction tubing. Change as needed. Check plastic connectors and parts for wear. Replace as needed.

Clean water recirculator and acid rinse the cooling water lines. Refill with fresh DI water. Check argon line filter. Replace as needed.

All analytical runs are recorded in the "ICP Instrument Run Log," with the analysis date, client identification, digestion page, instrument protocol, initials, and any relevant comments. All initial calibration data together with accepted values are stored in "Calibration Files". For each analytical batch, the documents are maintained for a period not less than one year. Initial and/or Continuing Calibration Verification data is produced as a raw data printout. Calibration Update data ir produced as a raw data printout. All analytical and laboratory QC samples data is produced as a raw data printout.

8.3.4 Cold Vapor Atomic Absorption Spectroscopy - Mercury (Hg)

Mercury is analyzed by an atomic absorption procedure that differs from flame and graphite furnace atomic absorption spectroscopy (AAS). Whereas flame and graphite furnace AAS rely on heat to break chemical bonds and to atomize the elements of interest, the cold vapor mercury method, as developed by Hatch and Ott (1968), takes advantage of elemental mercury's high vapor pressure. In this procedure, divalent mercury (Hg^{2+}) in aqueous samples (either water samples or tissue or sediment digests) is reduced to the elemental state (Hg^{0}) by a strong

reducing agent (stannous chloride). The fraction of Hg^0 that enters the gas phase is introduced into an atomic absorption cell, where light produced by a separate mercury vapor lamp is absorbed by the free Hg atoms. The amount of mercury in the sample is determined by comparing light absorption of the sample with that of calibration standards.

"Apparatus, Materials, and Reagents - Mercury"

- **Balance**: Top loading with accuracy of 0.001 g.
- **Microliter pipettes**: 1000-, 500-, 300-, 200-, 100-, 50-, 25- and 10-µL capacity.
- **Digital diluter** fitted with an appropriate syringe to deliver 0.04 mL of solution repeatedly.
- **Vortex genie variable speed vortex**
- **Reagent water**: Reagent water contains no analytes above the method detection limit. Reagent water is produced by redistilling water in a quartz still.
- **Nitric acid**: Baker Ultrex Grade or equivalent, stored in Teflon bottle. A 0.2 M solution is prepared by diluting 13 mL of concentrated HNO_3 to 1 L.
- **Hydrochloric acid**: Baker Ultrex Grade or equivalent, stored in Teflon bottle or original glass bottle.
- **Sulfuric Acid Reagent Grade**
- **Calibration Standard**: The calibration solution is made from a commercially available reference standard and dilute nitric acid. Serial dilutions of a 1000 ppm Hg stock solution are made with 0.2 M HNO_3.
- **Matrix Recovery Spiking Solution**: The matrix spiking solution is made similarly to the calibration standard solution but a more concentrated solution is needed. The concentration of this solution is determined such that the addition of a 0.2-0.5 mL volume will cause a 100% increase in observed concentration or 5 times the minimum detection limit, whichever is greater.
- **Stannous Chloride**: A 10% Sn^{2+} solution is used to reduce Hg^{2+} to Hg^o. It is made by adding 10 g $SnCl_2$ to 100 mL of 0.5 N H_2SO_4 prepared by adding 1.4 mL of conc. H_2SO_4 slowly to 100 mL of reagent water. Any Hg contamination can be removed by stirring this solution overnight, allowing Hg^o to escape to the atmosphere.
- **Sulfuric acid conc**.: Reagent grade of low mercury content
- **Nitric acid conc**.: Reagent grade of low mercury content
- **Stannous Sulfate**: Add 25 g stannous sulfate to 250 mL of 0.5 N sulfuric acid. This mixture is a suspension and should be stirred continuously during use.
- **Sodium Chloride-Hydroxylamine Sulfate Solution**: Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in distilled water and dilute to 100 mL.
- **Potassium Permanganate**: 5% solution, w/v. Dissolve 5 g of potassium permanganate in 100 mL of distilled water.
- **Potassium Persulfate**: 5% solution, w/v. Dissolve 5 g of potassium persulfate in 100 mL of distilled water.
- **Stock Mercury Solution**: Dissolve 0.1354 g of mercuric chloride in 75 mL of distilled water. Add 1.0 mL of nitric acid and adjust the volume to 100.0 mL. 1.0 - 1.0 mg Hg.
- **Working Mercury Solution**: Make successive dilutions of the stock mercury solution to obtain a working standard containing 0.1 µg/mL. This working standard and the dilution of the stock mercury solutions should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot.

8.3.4.1 Sample Preparation

Weigh a representative 0.2 g portion of wet sample and place in the bottom of a BOD bottle. Add 5 mL of concentrated sulfuric acid and 2.5 mL of concentrated nitric acid mixing after each addition. Heat two minutes in a water bath at 95ºC. Cool, add 50 mL of distilled water, 15 mL of potassium permanganate solution and 8 mL of potassium persulfate solution to each sample bottle. Mix thoroughly and place in the water bath for 30 minutes at 95ºC. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate. Add 55 mL of distilled water. Treating each bottle individually, purge the head space of the sample bottle for at least one minute and add 5 mL of stannous sulfate and immediately attach the bottle to the aeration apparatus. Continue as described.

An alternate digestion procedure employing an autoclave may also be used. In this method 5 mL of conc. H_2SO_4 and 2 mL of conc. HNO_3 are added to the 0.2 g of sample. Five (5) mL of saturated $KMnO_4$ solution and 8 mL of potassium persulfate solution are added and the bottle is covered with a piece of aluminum foil. The sample is autoclaved at 121ºC for 15 minutes. Cool, make up to a volume of 100 mL with distilled water and add 6 mL of sodium chloridehydroxylamine sulfate solution to reduce the excess permanganate. Purge the headspace of the sample bottle for at least one minute and continue as described.

8.3.4.2 Instrument Analysis

An example of a cold vapor mercury analyzer is an LCD Model 1235 uv Monitor equipped with a 30 cm path length absorption cell and operating at the 254 nm wavelength. The instrument has an attached chart recorder operating at the appropriate mV range to generate a full scale deflection with the highest standard used in the calibration.

Like many cold-vapor procedures this technique involves the use of a gas stream to strip Hg from the reaction vessel. The Sn^{2+} reductant is injected into the flask with the diluter. A small volume of sample or standard solution (0.5-2.0 mL) is introduced to the bottom of the 15 cc flow-through reaction chamber by vacuum. The flask is vortexed continuously to maximize contact. Contact of Hg^{2+} with the tin results in the reduction of Hg^{2+} to Hg^{o}. This produces an equilibrium distribution of Hg between the solution volume and the head space in the flask. The head space is drawn into the absorption cell of the UV Monitor by vacuum, and the resulting absorption of light by elemental Hg atoms results in a deflection of the recorder pen.

Because elemental mercury is distributed between the aqueous and gas phases, it is important that similar volumes of samples and standards are added to the reaction chamber at the beginning of the procedure. Since there is a limited volume available in the chamber, the amount of Hg in the gas phase is dependent upon the total amount of Hg available and on the relative volumes of liquid and air in the flask.

8.3.5 Calculations

Calculations are based upon measurements of peak height of samples, standards, and blanks, and are based on the following formula:

$$Hg = (PH_{spl} \text{ x } DF_{Hg}) - (PB) \text{ x slope x } DF_{dig'n} \div 1000$$

where:

Hg = the final mercury concentration in units of µg Hg per gram wet or dry weight of tissue or sediment, or ng Hg per mL of aqueous sample,

PH_{spl} = the peak height of the sample, in mm,

DF_{Hg} = the dilution factor needed to dilute samples to a concentration where they can be analyzed on the "0.02" range scale (i.e., to a level ~2 ppb)

PB = the peak height of the procedural blanks analyzed with the current batch of samples, in mm,

Slope = the slope of the calibration curve, with units of ppb Hg/m,

$DF_{dig'n}$ = the dilution factor resulting from final volume for digestion of the samples, with units of mL/g or mL/mL of aqueous sample.

Reporting units are µg/g (dry or wet weight) on µg/mL. The minimum method performance standard for the method is dependent upon the dilution factor resulting from digestion of the tissue or sediment sample. Assuming a typical dilution factor of 175 and normal blank levels and instrumental sensitivity, the minimum method performance standard is 0.01 µg/g Hg in a sample. The standard curve is acceptable if the linear regression of the standards has a variation of r2□0.995 or better. Results are reported to the number of significant figures that matches the number of such figures in the peak height. Results are reported to two (2) significant figures for samples with Hg peaks <100 mm, and to three (3) significant figures for samples with Hg peaks >100 mm.

Blank levels for mercury should be no more than 2x the method detection limit (MDL). If the level is above this limit, the analysis is repeated and the reanalysis data is reported if it meets the criterion. If insufficient sample is available, the data are reported with a blank correction and flagged as such. Blank spike recoveries are judged acceptable when it is within 80-120% of the true value. If the recovery is outside these limits, the analysis is repeated and the reanalyzed data is report if it meets the criterion. If more than 10% of the blank spikes in a delivery set are deemed unacceptable, then samples corresponding to these analytical batches are redigested.

Quality control samples are processed in a manner identical to actual samples. One method blank is analyzed with every 20 samples or with every sample set, whichever is more frequent. Blank levels should be no more than 2x method detection limit (MDL). If blank levels are > 2x MDL, the analysis is repeated and the data reported if QC criteria are met. If blank results are still > 2x MDL, the sample set is reprocessed after the source of contamination is isolated. If insufficient sample is available, data are reported with a blank correction and flagged as such.

Reference materials analyzed with the samples are matched as closely as possible to sample composition and expected trace metal concentration. Reference materials currently in use are listed below. Reference materials are deemed acceptable if the concentration is within ±20% of the certified value (if this is ≥2x MDL) or within the 95% confidence interval certified, whichever is greater. If the recovery is outside these limits, the analyses are repeated and the re-analyzed data is reported if it meets the criterion.

One reference material is analyzed with every 20 samples or with every sample set. Sediment or water certified reference materials, if available, and as closely matching the sample set as possible, are analyzed with each sample set. Reference materials can include:

Sediment samples:
- BEST-1 (NRCC, Canada),
- BCSS-1 (NRCC, Canada),
- MESS-2 (NRCC, Canada), and
- Estuarine sediment, #1646 (NIST, U.S.).

Reference material is deemed acceptable if the results are within 20% of the certified value (if this value is ≥2x MDL) or within the 95% confidence interval, whichever is greater.

Spikes are deemed valid if the analyte concentration of the sample is <2x MDL and the spike concentration is doubled or increased by ≤4x the MDL, whichever is greater. Only valid spikes can be judged acceptable or unacceptable. A valid spike is acceptable if the recovery is between 75-125%. If the recovery is outside these limits, the analysis is repeated and the reanalysis data is reported if it meets the criterion. If more than 10% of the spiked samples in a delivery set are deemed unacceptable then samples corresponding to these analytical batches are redigested. If this does not improve the recovery the samples are flagged as having a matrix dependent interference. Matrix spikes are not evaluated if the spike concentration is less than 10% of the sample concentration.

One matrix spike is analyzed with every 20 samples or with every sample set. Matrix spikes are used to investigate possible interferences which may result in either signal enhancement or suppression. Spiked and unspiked sample concentrations are compared and spike recovery is calculated according to the equation:

$$\text{Spike Recovery (\%)} = \frac{C_{spkd\ spl} - C_{spl}}{C_{spk}\ x \frac{V_{spk}}{V_{spl}}} x\,100$$

where:

$C_{spkd\ spl}$ = the concentration observed in the spiked sample,
C_{spl} = the concentration observed in the unspiked sample,
C_{spk} = the concentration of the spike,
V_{spk} = the volume of the spike (in mL), and
V_{spl} = the volume of the sample (in mL).

Spikes are deemed valid only if the analyte concentration of the sample is <2x MDL and the spiked concentration is doubled or increased by ≥4x the MDL, whichever is greater. Only valid spikes can be judged acceptable or unacceptable. When spike concentration is less than 10% of the sample concentration, calculations for matrix spikes are not performed.

All duplicate analyses are reported. Duplicates are judge for acceptability using relative percent difference (RPD):

$$RPD = |a - b| / x\,100$$

a = first analyte concentration; b = duplicate analyte concentration; c = (a + b)/2

RPD ≤ 20% (or other established value) is considered acceptable for samples with analyte concentrations that are greater than 2x the MDL. If the RPD is outside these limits, the analysis is repeated and the reanalysis data is reported if it meets the criterion. If the criterion cannot be met but other duplicate analyses are acceptable, then the data is flagged as being inhomogeneous.

One duplicate sample is analyzed with every 20 samples or with every sample set. Inhomogeneous samples may result in greater variability between duplicates. Experience has indicated that reference materials are more homogeneous than samples and thus comparison of the RPD of duplicates for a) reference material duplicate analyses, b) sample duplicate analyses and c) duplicate analyses from single digestion solutions gives an indication of a) total analytical variability (i.e., processing + instrumental variability), b) the sum of analytical variability and natural sample inhomogeneity, and c) instrumental variability. For standard QC purposes, "duplicate analysis" consists of preparation and analysis of duplicate sample aliquots. An RPD of ±20% is deemed acceptable for valid sample duplicate analyses (i.e., having analyte concentrations 2x MDL).

One blank spike laboratory control sample, a method blank fortified and carried through the digestion procedure, is analyzed with every 20 samples or with every sample set, whichever is more frequent. Blank spikes are analyzed to identify any digestion interferences, and to evaluate the performance of the analysis. Spike results are deemed acceptable when recoveries are within 80 to 120% of the known concentration.

The entire suite of calibration standards is analyzed at the beginning and end of each analytical batch. In addition, one or more calibration standards are analyzed after every 10 samples in order to observe instrumental drift and resultant change in sensitivity. If these differ from those analyzed earlier by >5%, they are re-analyzed. If there is still a difference, the system is checked for leaks, partially blocked tubing, etc. CCV % recovery is usually 85-115%.

The mercury analyzer is extremely stable and the sources of sensitivity changes are generally either flow-related (leaks, clogging) or due to a standard problem (e.g., introduction of only a minute amount of $SnCl_2$ will lead to a significant loss of Hg from the standard) or a deteriorated $SnCl_2$ solution.

If this remediation restores the original sensitivity, samples analyzed immediately before and after the last acceptable calibration check are re-analyzed. When drift cannot be corrected, samples are calibrated against standards analyzed at the beginning and end of each group of ten samples. When these standards differ significantly from one another, the samples are re-analyzed.

8.4 Total Petroleum Hydrocarbons

In cases where it is suspected that soils and sediments may be contaminated with petroleum hydrocarbons, a hierarchy of methods can be used. The simplest procedure is to extract the dry soil or sediment with methylene chloride to isolate the petroleum hydrocarbons and weigh the extract to determine what is operationally defined as "oil and grease". This measurement may, however, include non-petroleum hydrocarbons (e.g., vegetable oils, animal fat, waxes, soaps, greases, etc.). The method is also not applicable for light petroleum hydrocarbons. For example, only half of the components of gasoline would be determined by the oil and grease method.

If more specificity is required, the methylene chloride extract can be processed on a silica column that will remove many non-petroleum hydrocarbon interferences. This total petroleum hydrocarbon is then a "cleaned-up fraction" and can then be determined by weighing (gravimetry), spectroscopy or by the more specific method of gas chromatography (GC). The spectrophotometric and GC methods are more specific but also require more expensive instruments.

8.4.1 Sample Preparation

Sediment and soil samples (10-15 grams) are soxhlet extracted with methylene chloride. The extract is placed in a 125 mL flat bottom flask with a 3-balled Snyder column and placed in 70ºC water bath and exchanged to one (1) mL of hexane.

"Apparatus, Materials, and Reagents - Total Petroleum Hydrocarbons"

- **Soxhlet Extractor**, capable of holding 10-15 grams of sediment.
- **Column chromatography apparatus** for silica gel cleanup.
- **Balance** capable of weighting µg aliquots.
- **Water Bath** or system capable of reduced pressure distillation.
- **Flask**, boiling, 125 mL (Corning No. 4100 or equivalent).
- **Methylene chloride**.
- **Silica gel**, 60-200 mesh, Davidson Grade 950 or equivalent. Should contain 1-2% water as defined by residue test at 130°C. Adjust by overnight equilibration if needed.

The recovered extracts can be quantified for total hydrocarbon content as well as specifically for aromatic hydrocarbons.

Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials shall be demonstrated to be free from interferences under the conditions of the analysis by running

method blanks initially and with a set of samples. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Glassware and, where possible, reagents are cleaned by solvent rinse or baking at 450ºC for a minimum of 1 hour.

8.4.2 Gravimetry

An aliquot of the extract is weighed. Oil and grease is calculated as follows:

$$\text{mg/g of oil and grease} = \frac{A \frac{V_1}{V_2}}{g}$$

where:

A = weight of aliquot (mg)
V_1 = total volume of extract (usually 1000 µL)
V_2 = volume of extract weighed (usually 20 µL)
g = weight of sample extracted (g)

The extract can be processed through a silica column. The solvent evaporation and an aliquot weighed to determine the total petroleum hydrocarbon by the equation provided above. As in the case of oil and grease, the parameter of petroleum hydrocarbons is operationally defined by the method.

8.4.3 Infrared Spectrophotometry

The sample extract in hexane is analyzed on an infrared spectrophotometer. This allows for the determination of oil and grease or total petroleum hydrocarbons.

"Apparatus, Materials, and Reagents - Spectrophotometry"

- **Infrared Spectrophotometer**, scanning. Non-scanning instruments may also be used but can be subject to positive interferences in complex chemical wastewaters.
- **Cells,** 10 mm, 50 mm, and 100 mm path length, sodium chloride or infrared grade glass.
- **Volumetric Flasks** 10 and 100 mL.
- **Water Bath** or system capable of reduced pressure distillation.
- **Calibration Mixtures**

  Reference oil: Pipet 15.0 mL n-hexadecane, 15.0 mL isooctane, and 10.0 mL chlorobeneze into a 50 mL glass stoppered bottle. Maintain the integrity of the mixture by keeping stoppered except when withdrawing aliquots.

  Stock Standard: Pipet 1.0 mL reference oil into a tared 200 mL volumetric flask and immediately stopper. Weigh and dilute to volume with hexane.

  Working Standards: Pipet appropriate volumes of stock standard into 100 mL volumetric flasks according to the cell path length to be used. Dilute to volume with hexane. Calculate concentration of standards from the stock standard.

The sediment or soil extract (see Section 8.5.1) before silica column clean-up (oil and

grease) or after silica clean-up (TPH) is diluted to 10 mL or 100 mL.

Select appropriate working standards and cell path length according to the following table of approximate working ranges:

Path Length	Range
10 mm	2-40 mg
50 mm	0.4-8 mg
100 mm	0.1-4 mg

Scan standards and samples from 3200 cm^{-1} to 2700 cm^{-1} with hexane in the reference beam and record the results. The absorbances of samples and standards are measured by constructing a straight baseline over the range of the scan and measuring the absorbance of the peak maximum at 2930 cm^{-1} and subtracting the baseline absorbance at that point. For an example of a typical oil spectrum and baseline construction (see Gruenfeld 1973). Non-scanning instruments should be operated according to manufacturer's instructions although calibration must be performed using the standards described above. If the absorbance exceeds 0.8 for a sample, select a shorter pathlength or dilute as required.

Use a calibration plot of absorbance vs. mg oil prepared from the standards to determine the mg oil in the sample solution.

Calculation: mg/g total oil and grease = (R x D)/g

where:

R = response factor for oil in solution, determined from calibration plot, in milligrams
D = extract dilution factor, if used.
g = grams of sediment extracted.

8.4.4 Gas Chromatography

The determination of total petroleum hydrocarbons (TPH) can provide an initial screening method to determine the extent of petroleum, coal tar, creosote, refined oil, etc. contamination. This method is applicable to sediments, soils, water, wastewater, seawater, and snow after sample extraction. If there is a specific product spill (e.g., diesel fuel), that product can be used as a standard, making the method specific for that product. The detection limit for this method is 200 μg/g or mg/L. When this method is used to analyze samples for which there is no reference diesel oil, diesel oil identification should be supported by at least one additional qualitative technique, i.e., gas chromatograph/mass spectrometer (GC/MS). The detection limit of this method is usually dependent upon the presence of other oils in the sample. Excluding interferences, estimated detection limits of 200 mg/kg of total oil and 100 mg/kg of diesel can be obtained.

Samples are extracted as described in Section 8.5.1 The extract is dried by passage through sodium sulfate. A subaliquot of the extract is evaporated to dryness, and the total amount of oil in the sample is determined gravimetrically. The oil, together with a measured

volume of internal standard, is redissolved in a known volume of methylene chloride, and an aliquot is injected into a gas chromatograph (GC). The components of the oil are separated by the GC and detected using a flame ionization detector (FID). Identification of diesel oil (qualitative analysis) is performed by comparing the pattern of GC peaks (retention times and intensities) from the sample extract with the pattern of GC peaks from a reference diesel oil sample. Identification of diesel oil is established when the reference diesel and sample patterns agree per the criteria in this method. Quantitative analysis of diesel oil is performed using an internal standard technique.

There is no standard diesel oil. Oil components, as seen by GC/FID, will differ depending upon the oil source. For rigorous identification and quantification of diesel oil in a fluid sample by GC/FID, the chromatographic pattern from the diesel oil should be matched with the chromatographic pattern from a reference standard of the same diesel oil suspected to be in the sample.

"Apparatus, Materials, and Reagents - TPH-Gas Chromatography"

- **Bottle**: Boston round wide-mouth jar with PTFE-lined screw-cap (Sargent Welsh S-9184-72CA, or equivalent). New bottles are used as received with no further cleaning required.
- **Glass wool**: Pyrex (Corning 3950, or equivalent). Solvent-extracted or baked at 450ºC for a minimum of 1 hour.
- **Separatory funnel**: 60-mL with PTFE stopcock.
- **Drying column**: Pyrex chromatographic column, 400 mm long by 15 to 20 mm i.d., equipped with coarse-glass frit or glass-wool plug.
- **Glass filtering funnel**: Crucible holder (Corning No. 9480, or equivalent).
- **Spatulas**: Stainless steel or PTFE.
- **Evaporation flask**: 500-mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012).
- **Concentrator tube**: l0-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.
- **Snyder column**: Three-ball macro (Kontes K-503000-0232, or equivalent).
- **Snyder column**: Two-ball micro (Kontes K-469002-02 19, or equivalent).
- **Boiling chips**: Teflon, pre-rinsed with methylene chloride.
- **Glass or silicon carbide**: Approximately 10/40 mesh, extracted with methylene chloride and baked at 450ºC for a minimum of 1 hour.
- **PTFE** (optional): Extracted with methylene chloride.
- **Water bath**: Heated, with concentric ring cover, capable of temperature control (±2ºC), installed in a fume hood.
- **Sample vials**: Amber glass, 1- to 5-mL with PTFE-lined screw- or crimp-cap, to fit GC autosampler.
- **Analytical balance**: Capable of weighing 0.1 mg. Calibration must be verified with class S weights each day of use.
- **Top-loading balance**: Capable of weighing 10 mg.
- **Sodium sulfate**: Anhydrous, ACS grade, granular.

- **Methylene chloride**: Nanograde or equivalent.
- **Reagent water**: Water in which the compounds of interest and interfering compounds are not detected by this method.
- **Internal standard**: Dissolve 1.0 g of 1,3,5-trichlorobenzene (Kodak No. 1801 or equivalent) in 100 mL methylene chloride. Store in glass and tightly cap with PTFE-lined lid to prevent loss of solvent by evaporation. Label with the concentration and date. Mark the level of the meniscus on the bottle to detect solvent loss.
- **Calibration standards**: Calibration standards are prepared from the same diesel oil expected to be in the sample; otherwise, No. 2 diesel oil is used.
- **Stock solutions of calibration standards**: Weigh the appropriate amount of oil into a tared l0-mL volumetric flask and dilute to volume with methylene chloride. Label each flask with the concentration and date.
- **Micropipette or microsyringe**, transfer 100 µL of each reference standard solution to a GC injection vial. Add 100 µL of the TCB internal standard to each vial and mix thoroughly. Calibration standards are made fresh daily to avoid solvent loss by evaporation.
- **QC standard**: Used for tests of initial and ongoing performance. Prepare a reference mud sample containing 20,000 mg/kg of diesel by adding 20.0 mg (±0.2 mg) of No. 2 diesel oil and 10.0 g (±0.1 g) of EPA Generic Mud No. 8 to a clean retort cup. Mix the mud and diesel oil thoroughly with a metal spatula.

The analytical system indicates a split injection, a capillary column, a temperature programmer with initial and final isothermal holds, and all of the required accessories, including syringes, analytical columns, gases, detector, and recorder. The GC Columns are 30 m (±5 m) long by 0.25 mm (±0.02 mm) i.d., 99% methyl, 1% vinyl, 1.0 µm film thickness, bonded-phase fused-silica capillary (Supelco SPB-l, or equivalent). The detector is flame ionization. This detector has proven effective in the analyses of drilling fluids for diesel oil. Guidelines for using alternative detectors are provided.

The GC data system can collect and record GC data, store GC runs in magnetic memory or on magnetic disk or tape, process GC data, compute peak areas, store calibration data including retention times and response factors, identify GC peaks through retention times, and compute concentrations. GC data shall be collected continuously throughout the analysis and stored on a magnetic storage device. The data system is used to record and maintain lists of response factors and multi-point calibration curve. Statistics on initial and ongoing performance shall be computed and maintained. The data system shall search, locate, identify, and quantify the compounds of interest in each GC analysis. Software routines shall be employed to compute and record retention times and peak areas. Displays of chromatograms and library comparisons are required to verify results.

Gas chromatographic operating conditions must be established. Verify that the GC meets the manufacturers performance criteria and the estimated detection limit. The gas chromatographic system is calibrated using the internal standard technique. Because each GC is slightly different, it may be necessary to adjust the operating conditions (carrier gas flow rate and column temperature and temperature program) slightly until the retention times are met.

Tabulate the peak area responses against concentration for each of the ten largest peaks in the chromatogram (excluding the solvent peak, the internal standard peak, and any peaks that elute prior to the internal standard peak). Calculate response factors (RF) for each peak using the following equation:

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)} \qquad \text{Equation 1}$$

where:

A_s = Area of the analyte.
C_{is} = Concentration of the internal standard, in mg/kg.
A_{is} = Area of the internal standard peak.
C_s = Concentration of the peak to be measured, in mg/kg.

If the RF is constant (<15% CV) over the calibration range, the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is} vs. RF.

Calibration verification: For each peak, the average RF or a point on the calibration curve shall be verified on each working day by the measurement of one or more calibration standards. If the RF for any peak varies from the RF obtained in the calibration by more than $\pm$ 15%, the test shall be repeated using a fresh calibration standard. Alternatively, a new calibration curve shall he prepared.

Combined response factor: To reduce the error associated with the measurement of a single peak, a combined response factor is used for computation of the diesel oil concentration. This combined response factor is the sum of the individual response factors as given in Equations 2 or 3:

$$\text{RF Combined } RF(1) + RF(2)... + RF(n) \qquad \text{Equation 2}$$

$$RF\,\text{Combined} = \frac{[A_{s(1)} + A_{s(2)} \cdots + A_{s(n)}](C_{is})}{(A_{is})(C_s)} \qquad \text{Equation 3}$$

where:

n = Number of individuals peaks
$A_{s(l)} \ldots A_{(n)}$ = Areas of the individual peaks
C_{is} = Concentration of the internal standard, in mg/kg
A_{is} = Area of the internal standard peak
C_s = Concentration of the peak to be measured, in mg/kg

The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described. The analyst is permitted to modify this method to improve separations or lower the costs of measurements,

provided all performance requirements are met. Each time a modification is made to the method, the analyst is required to achieve the estimated detection limit. Analyses of spiked samples are required to demonstrate method accuracy. Analyses of duplicate samples are required to demonstrate method precision. Analyses of blanks are required to demonstrate freedom from contamination. The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the QC standard that the analysis system is in control. The laboratory shall maintain records to define the quality of data that is generated.

8.5 Polycyclic Aromatic Hydrocarbons

Assessment of the environmental impact of hydrocarbons can require the measurement of substituted and unsubstituted polynuclear aromatic hydrocarbon compounds (PAH) at trace levels (ng/g or μg/L). These standard operating procedures provide precise and accurate methods to quantitatively determine petroleum related compounds in snow (water) and soils/sediments. Acidified water samples are serially extracted with methylene chloride in a separatory funnel. The extract is dried and concentrated. An optional alumina column cleanup step is provided for use before the instrumental analysis to remove matrix interferences. The protocol is designed for 2-L water samples, but water samples of other sizes may be collected and extracted by appropriately adjusting the volume of methylene chloride used for the extraction.

8.5.1 Sample Preparation

The following procedure is a modification of EPA SW846 Method 3540 and Method 3611 for Soxhlet extraction of the petroleum hydrocarbon compounds from sediments and extract purification by alumina column chromatography. Ten to thirty grams of chemically dried or oven dried sediment is Soxhlet extracted with methylene chloride and the extract is concentrated and purified using EPA Method 3611 alumina column purification to remove matrix interferences. The alumina purification procedure is usually required prior to analysis. The aliphatic and aromatic fractions are collected in a single fraction. The purified extract is then submitted for analysis of aliphatic and aromatic hydrocarbons.

Sediment should be collected in precleaned glass jars, or core liners and frozen (-20°C) in the field. Sediment samples are shipped frozen to the laboratory and stored at -20°C until analysis. After subsampling excess sample is archived at -20°C in the dark. Sample extracts are stored in the dark at or below 4°C.

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware (i.e., freeze dryers) that lead to false positives in GC/FID or GC/MS detection. All materials used in this method are routinely demonstrated to be free from interferences by processing procedural blanks consisting of combusted sand with water added through the entire analytical procedure including freeze drying (one blank per 20 samples or each batch, whichever is more frequent). Field blanks are sometimes also analyzed to see if contamination has occurred during sampling. These blanks, when available, will be run with their respective sample batches through the entire analytical procedure.

Matrix interferences result from co-extraction of compounds other than the analytes of interest. Elemental sulfur and naturally occurring materials can cause interferences in the

analysis of sediment extracts. Alumina cleanup with activated copper is used to purify the sample prior to analysis.

Glassware is cleaned by detergent (micro cleaning solution) washing with water and rinsing with tap water. The glassware is then combusted in a muffle furnace at 400°C for at least 4 hours. Solvent rinses of acetone to dry followed by methylene chloride may be substituted for the muffle furnace heating when determined to be appropriate by the analyst. After drying and cooling, glassware is sealed and stored in a clean environment to prevent the accumulation of dust or other contaminants. Stored glassware is maintained capped with combusted aluminum foil.

"Apparatus, Materials, and Reagents - PAH"

- **Glass Jars**: 250 mL or 500-mL glass jars, or other suitable containers.
- **Vials**: 1-mL to 7-mL glass vials with Teflon-lined caps.
- **Glass Funnels**
- **Flat Bottom Flasks**: 250 and 500 mL.
- **Soxhlet Extractor Flasks**: 40 mm ID and condensor.
- **Thimbles**: Aluminum, medium or coarse, 44-mL round bottom.
- **Concentrator Tube**: Kuderna-Danish - 25 mL, graduated. Ground glass stoppers are used to prevent evaporation of extracts.
- **Snyder Column**: Kuderna-Danish - Three ball column.
- **Micro Reaction Vessels**: 2.0 mL or 1.0 mL autosampler vials with crimp cap septa.
- **Chromatographic Column**: 300-mm x 10-mm inner diameter, with Pyrex glass wool at bottom and Teflon stopcock.
- **Analytical Balance**: Capable of weighing to 0.0001 mg.
- **Analytical Balance**: Capable of weighing to 0.1 g.
- **Water Bath**: Heated to 60°-70°C.
- **Teflon Boiling Chips**: Solvent extracted.
- **Syringes**: 10 or 25 µL.
- **Disposable Glass Pasteur Pipettes**: 1-mL.
- **Pyrex Glass Wool**: Combusted to 400°C.
- **Nitrogen Gas Evaporation Unit**

Note: Volumetric glassware for sample measurement or introduction of internal standards must be calibrated.

- **Reagent Water**: Reagent water contains no analytes above the method detection limit.
- **Sodium Sulfate**: (ACS) Granular, anhydrous (purified by heating at 400ºC for 4 h in a shallow tray or other suitable method).
- **Solvents**: Hexane, methylene chloride (pesticide quality or equivalent).
- **Alumina**: Neutral 80-325 MCB chromatographic grade or equivalent. Combust alumina at 400°C for 4 hr and store at 120°C prior to use.
- **Activated Copper Turnings**
- **Granular Copper**
- **Surrogate Spiking Solutions**
- **Matrix Spike Standard**
- **Internal Standard Solution**

A subsample (~1 g) is weighed, oven-dried and reweighed to obtain percent moisture. The rest of the sample is also oven- or chemically-dried unless a frozen archive sample is needed. The oven-dried sample is homogenized by mortar and pestle and an aliquot (10 g) is removed and accurately weighed for analysis. Alternatively, a weighed aliquot (10-30 g) of the sample is chemically-dried by mixing the sample with 30-100 g of sodium sulfate, adding additional sodium sulfate until the sample is dry, free-flowing and homogeneous. Core samples or sections are processed by extruding the frozen core after slightly warming the outside liner. Add the sample to an extraction thimble and place the thimble into the Soxhlet holder. A ball of copper wire of about 3 centimeters diameter is cleaned in 10% HCl, then water, methanol, and methylene chloride. One ball is placed in each 250 mL extraction flask. Add 150 mL of methylene chloride to the extraction flask containing 1 or 2 boiling chips. Add 50 mL of methylene chloride to the sediment in the thimble. Add surrogates to the CH_2Cl_2 wetted sediment in the thimble. Spike with PAH surrogates. Attach the 250 mL flat bottom flask and extract the sample for 4 to 8 hours. Recycling should occur every 4 minutes. If necessary, filter and dry the extract with glass wool and sodium sulfate. Concentrate the extract by Kuderna-Danish techniques to 4-5 mL. Transfer to a 25 mL concentrator tube. Rinse the flat bottom flask three times with 2 mL of methylene chloride and transfer the rinse to the concentrator tube. Concentrate the extract and exchange the solvent to 2.0 mL hexane.

Alumina column clean-up is usually recommended. Place a plug of combusted glass wool in the bottom of the glass chromatographic column. Add 1-1/2 centimeters of combusted quartz sand to cover the glass wool. Fill the reservoir with hexane and add 10.0 g alumina. Gently tap the column to distribute the alumina evenly. Alternatively, a slurry of alumina in hexane may be used to pack the column. Allow the alumina to settle and then add 1.0 g of anhydrous sodium sulfate on top of the alumina and 10.0 g of activated copper. Drain the hexane and elute the column with 50 mL of hexane. Drain the column until the head of the liquid in the column is just above the sodium sulfate layer. Transfer the sample extract onto the column. Rinse the extract vial with 1 mL methylene chloride and add it to the column immediately. To avoid overloading the column, it is suggested that no more than 200 mg of extract should be placed on the column. Add 100 mL methylene chloride, and elute at a flow rate of 2 mL/min. Collect the effluent in a 250 mL flat bottom flask. The collected fraction contains aliphatic and aromatic hydrocarbons. Concentrate the extract to 0.5 mL hexane.

Reporting units are ng/g (wet or dry weight). The detection limit for the method is 4,000 ng/g for the unresolved complex mixture, 3 ng/g for individual alkanes and isoprenoids, and 1 ng/g for individual PAH compounds based on dry sample weight. The performance can be adjusted by decreasing final sample volume, increasing sample amount and/or increasing volume injected.

8.5.2 Spectrophotometry

A semi-quantitative estimate of aromatic hydrocarbons can be obtained with the ultraviolet/fluorescence procedure but the analyst is reminded of the limitations due to possible interference of non-hydrocarbon organic compounds. The procedures described here are from United Nations Environment Program (1992).

The fluorometer must be capable of synchronous scanning of excitation and emission wavelengths and be connected to a recorder or data system. The fluorometer is first tested for its signal to noise ratio and the accuracy of the monochromators by following the instructions in the instrument manual. This procedure is briefly described below. The hexane used for sample preparation is then tested for its contribution to blank signals. Aliquots of sample fractions in hexane are then scanned. Emission scans and synchronous excitation/emission scans are recorded and evaluated for the type of aromatic hydrocarbons in the samples. Standard response curves of fluorescence intensity versus concentration are generated for appropriate oil standards and for the standard aromatic hydrocarbon, chrysene. The sample fractions are diluted to give a reading within the linearly calibrated range of the fluorometer. Aliquots of the hexane solutions of blanks, standards, and samples are pipetted in turn into a quartz fluorometer cell. All blanks, standards, and samples should be run at identical instrument settings and conditions and using the same batch of pre-tested hexane.

The signal to noise ratio is tested as follows. Turn on the xenon lamp and allow instrument to warm up for about 30 minutes. Set controls:

mode:	ENERGY (electronic filtering mechanism turned off)
response:	NORM (2.0 sec)
GAIN switch:	NORM (750 V)

Carefully fill the 10 mm quartz cell with distilled water ensuring that there are no bubbles, and position the cell in the sample compartment. Set the following conditions:

EMISSION SLIT:	10 nm
EXCITATION SLIT:	10 nm
SAMPLE SHUTTER:	open
EMISSION WAVELENGTH:	397 nm
EXCITATION WAVELENGTH	350 nm
SCAN SPEED:	60 nm/min

By turning the coarse and fine SENSITIVITY knobs, deflect the recorder pen to about 80% of full scale at 397 nm (Range typically 3.0 or 1.0).

Set EMISSION WAVELENGTH dial at 300 nm. Record the emission spectrum of the distilled water from 330 nm to about 500 nm. Turn off the WAVELENGTH DRIVE and manually scan back to the emission maximum of the Raman band which should be at 397 nm. Record the noise level at this maximum. Measure the signal height of the Raman peak from the floating baseline to the peak height. Measure the height of the noise. The signal to noise ratio should be 80/1 or greater (Figure 8.1). Keep a running log of signal to noise ratios and hours of use on the xenon lamp. Replace the lamp when its signal to noise ratio is unsatisfactory. Note: Follow all precautions in the instrument manual for handling and aligning new lamps. Use gloves and never touch the lamps with your fingers. Wear protecting cobalt eye glasses if necessary to view a lighted UV lamp.

The instrument manual will have more tests for stability and wavelength accuracy. Follow these instructions to become familiar with the operation and performance of the fluorometer.

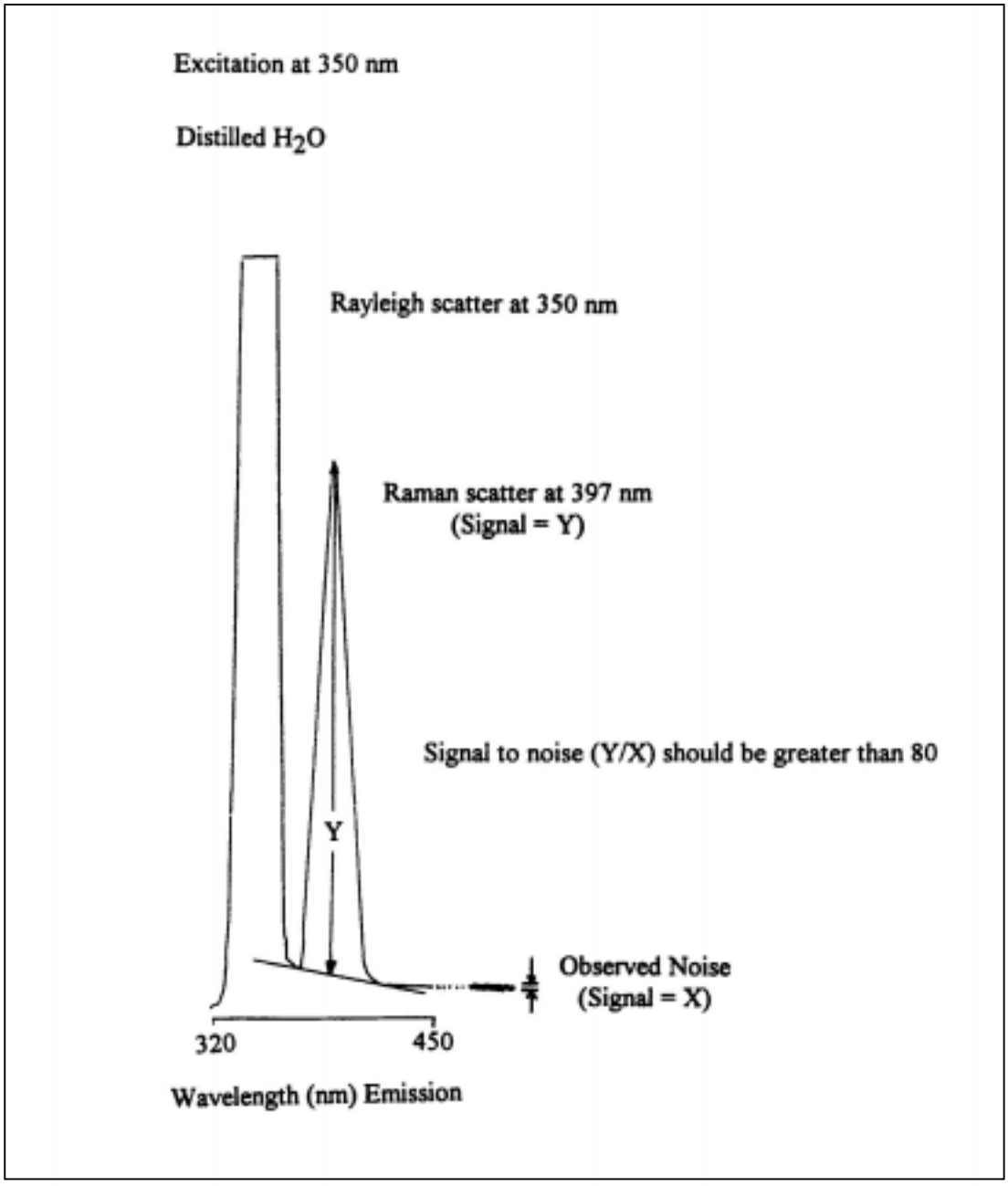

Figure 8.1. Rayleigh and Raman scatter plus signal to noise ratio (from UNEP 1992).

For the fluorescence analysis, samples are dissolved in hexane. The complete procedural blank value is acceptable for sample correction if its fluorescence reading does not exceed twice the fluorescence reading of the unconcentrated hexane.

One set of standards should be chrysene in hexane and some intercalibration programs have reported results in terms of chrysene units. Construction of this calibration curve will thus aid in the intercomparison of data sets. However, before the standard chrysene can be used for calibration, its correct emission spectrum must be verified. This is done by exciting the chrysene solution at 310 nm and recording its emission spectrum. The correct spectrum will show an emission maximum near 362 nm and 381 nm and a smaller maximum near 408 nm.

A hexane stock solution is prepared by dissolving 1l0 mg chrysene per 100 mL of hexane. Note that although the chrysene is quite soluble at this concentration, the kinetics of dissolution are slow. Thus the stock solutions must stand at least 12 hrs before dilution. From the stock solution a range of dilutions is prepared. The required range will depend on the sensitivity of the fluorometer. Under the conditions specified for the analysis, modern instruments will give a linear response up to a chrysene concentration of approximately 3 μg/mL. 20 mL glass scintillation vials with aluminum foil lined lids are useful vessels for the preparation of standard dilutions. The necessary volume of stock solution is pipetted into the preweighed vial which is partially filled with hexane. The volume is then brought up to 20 mL with hexane (1.0 mL of hexane weighs 0.6603 g).

Standard solutions should also be prepared from refined or crude oils. Oil standards are prepared in much the same way as the chrysene standards. The density of the oil in g/mL is determined by weighing a measured volume of oil. Then enough oil is transferred to the 100 mL volumetric stock flask to yield a concentration of approximately 0.5 mg oil per mL of hexane. This stock solution is then used to prepare the standard dilutions in the linear calibration range. This is usually in the 0 to 10 μg/mL range for most oils, but will vary depending on the aromatic content of the soil. Some oils, such as Kuwait crude, are difficult to dissolve and will require that the primary stock solution is much less concentrated than the average values given here. The oil standards are run in the fluorometer at the same sensitivity settings as the chrysene standards.

When reporting results in terms of oil units, an intercomparison ratio between the fluorescence intensity of the oil and that of chrysene should be calculated according to the formula:

$$R = \frac{\text{fluorescence intensity of chrysene}}{\text{fluorescence intensity of oil standard}} \times \frac{\text{concentration of oil standard in } \mu g/mL}{\text{concentration of chrysene standard in } \mu g/mL}$$

The choice of oils used for calibration and the wavelengths for the quantitative measurement will depend on the types of oil seen in samples from individual monitoring programs. The analyst should compile a “library” of spectra and response curves generated from the particular instrument used.

For qualitative identification of oil type based on the fluorescence of aromatic hydrocarbon components, a synchronous excitation/emission scan provides the best resolution with elimination of spectral interferences due to Rayleigh and Raman scattering. The excitation

monochomator is set 23 nm below the emission wavelength and the two monochromators are scanned synchronously (start with excitation at 237 nm and emission at 260 nm). Set the excitation monochromator slits (or band pass widths) at 10 nm and emission monochromator slits at 3 to 5 nm depending on instrument sensitivity. Set scan speed at 50 nm/min and recorder speed at 3 or 6 cm/min. Record synchronous spectra between 260 nm and about 525 nm emission. Examples of synchronous excitation-emission spectra generated from standard oils and extracts from sediment contaminated with oil, using a Perkin Elmer 650 instrument are shown in Figure 8.2. Notations for the emission region of single through five ringed compounds are taken from Popi et al. (1975). Inspection of these spectra indicate that diesel oil contains mostly substituted naphthalenes and no signal due to the higher ringed aromatics. Crude oils show maxima in higher wavelength regions. Microbial degradation of the side chains on the aromatic rings will shift emission maxima to shorter wavelengths (as much as 15 nm), whereas solution processes will preferentially remove the more soluble single and double ring components. Thus for accurate interpretation the analyst must be familiar not only with the characteristic spectra generated by standard oils, but also with the effects of weathering processes in altering the fluorescence spectra.

When the oil in samples has been matched as closely as possible to the available standards, then this standard can be used for constructing the calibration curve. First determine the optimum wavelengths for the quantitative measurement. This is achieved by fixing the emission wavelength and obtaining an excitation spectrum. The wavelength of optimum excitation is then chosen and fixed and an emission spectrum obtained. The most sensitive wavelengths would then be those empirically determined from the excitation and emission spectra. For average crude oils these optima are at 310 nm excitation and 360 nm emission. For diesel oil these optima are at 280 nm and 327 nm, respectively (280 nm/327 nm).

Calibration curves are constructed by setting the excitation and emission monochromators at 310 nm and 360 nm respectively for chrysene and most crude oils. The standard dilutions or stock aliquots are pipetted into the 10 mm cell and the fluorescence intensity (FI) measured. A plot of FI vs μg/mL should be constructed and the regression equation calculated over the linear range as per Figure 8.3. Calibrations should be obtained daily and a running log kept. As aliquots are removed from the stock vials, the vials should be reweighed to ensure no solvent loss, and hence no change in concentration. New dilutions should be prepared on a routine basis.

A quick alternative method for constructing calibration curves is prepare the standard oil solutions to a stock concentration of approximately 0.2 mg/mL for most medium weight crude oils or 1 mg/mL for less fluorescent oils such as diesel fuel. The calibration curve is then constructed by placing 2 mL of hexane into the cuvette and taking the blank reading at the appropriate wavelengths and instrument settings. Sequential 10 μL or 5 μL aliquots of the stock oil solutions are then added to the cuvette, the sample mixed and a reading taken after each addition.

The FI of each sample (total extractable lipids or the aromatic fractions of sample extracts) is determined and the concentration of oil determined from the standard curve. Samples must be diluted to produce readings in the linear region. This is easily done by the procedure of placing 2 mL hexane in the cell and adding sample extract in μL aliquots. The

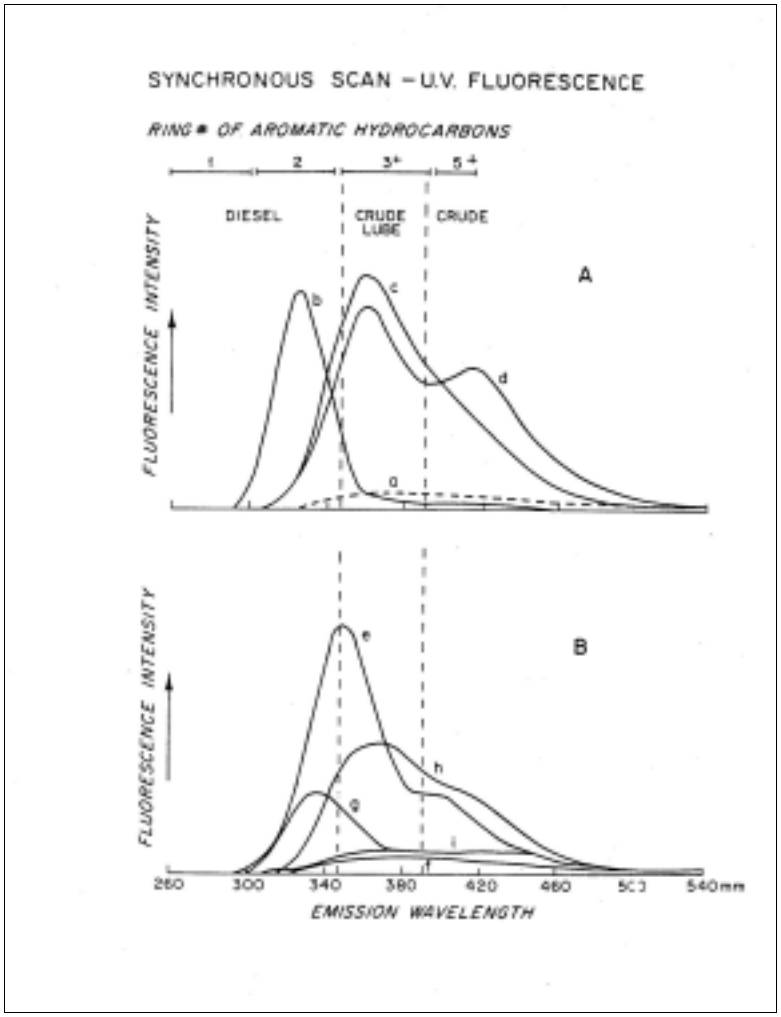

Figure 8.2 Synchronous excitation/emission fluorescence spectra of aromatic hydrocarbon fractions of: (A) source oils and (B) Environmental samples. Scan from 260 to 540 emission with excitation set 23 nm lower. Band pass 5 nm. (a) hexane blank, (b) diesel oil, (c) lube oil, (d) Gippsland crude oil, (e) mussels from refinery wharf, (f) clean mussels, (g) mussels from boat wharf, (h) sediments near refinery, and (i) clean sediments (from UNEP 1992).

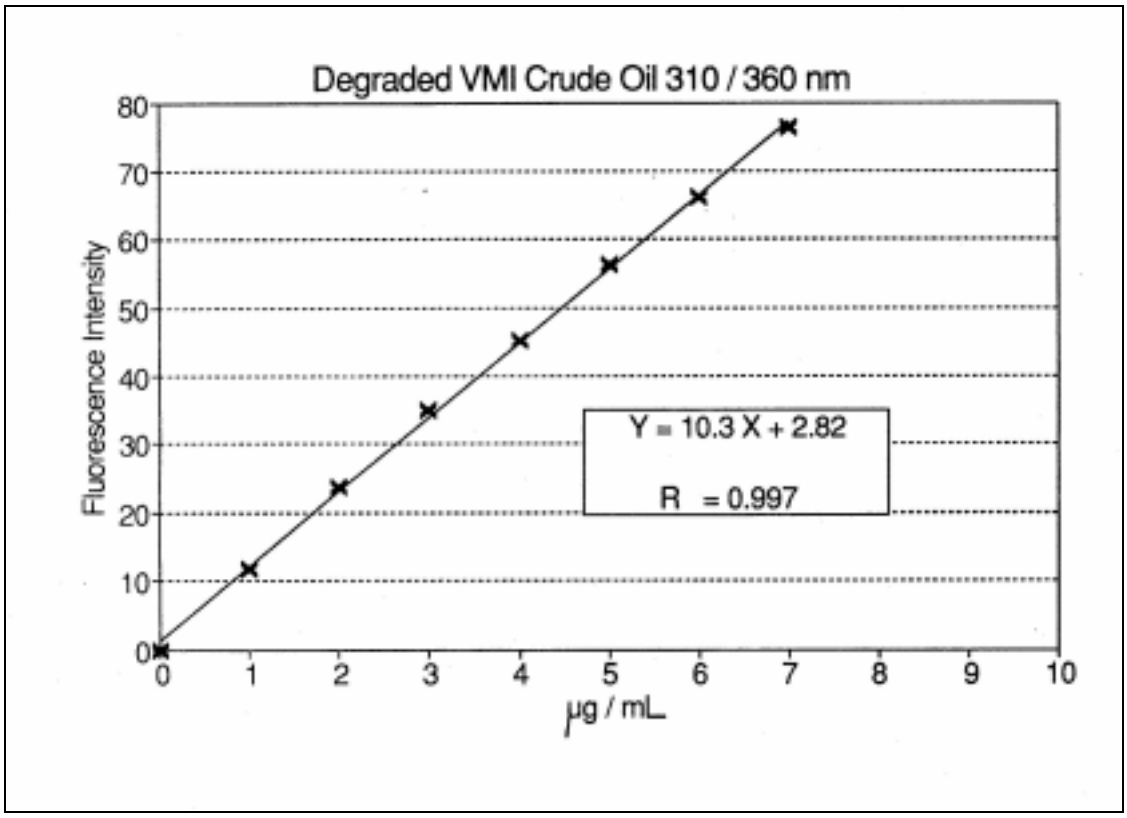

Figure 8.3. Calibration graph: degraded VMI crude oil 310/360 nm (from UNEP 1992).

sample dilutions must be recorded (dilution is total µL sample extract divided by the µL used for FI determination. If any correction must be applied for only a portion of the extract brought to this stage of the analysis, it must also be applied to the dilution). The semi-quantitative estimate of oil content is calculated from the regression line and expressed as µg/mL. Sample content is then calculated as follows:

$$\frac{\mu g\ oil\ (or\ chrysene)}{g\ dry\ weight} = \frac{\mu g}{mL}\ x\ 2\ mL\ x\ dilution\ x\ \frac{1}{g\ dry\ weight}$$

The results are reported in µg/g "chrysene or oil equivalents". If the sample extract has been fractionated into hydrocarbon classes, the intensity of fluorescence of each fraction will depend on the relative amounts of single through five and heavier ringed aromatics in the fractions. An example of the relative distribution of FI measured at 210 nm/360 nm between fractions from the column chromatography separation of a few standards oils are listed below:

	(F1)	(F2)	(F3)
Venez/Mex Ismus Crude Oil	4%	48%	20%
Gippsland Crude Oil	5%	53%	42%
Marine Diesel Oil	0%	93%	7%
Lubricating Oil	16%	44%	40%

The intensity of the emitted fluorescence is a function of the character of the fluorescent compounds in a sample, their concentrations, the dimension of the sample in the light path of the photometer, and also of the intensity of the excitation source.

There is a linear relationship between the sample concentration of fluorescing compounds and the intensity of the emitted light only within certain limits when the total absorbance of the sample is low. If the absorbance exceeds 0.05 absorbance units (less than 95% transmittance), FI will be reduced by the presence of any compound in the sample that can absorb either the excitation light or the emitted light. As this effect takes place in the sample cell, the disturbance is called an "inner filter effect". At high concentration this can be caused by the fluorescing compound itself. This is common in highly polluted sample extracts. Other compounds co-extracted with the oil may also cause a reduction in emitted light by "quenching". In this instance the energy is transferred to the co-extracted substances or "quenchers," rather than being emitted as a fluorescence emission.

The following tests can be used to test for inner filter effects. None of these experiments are fully conclusive and a good knowledge of fluorometry is necessary for their application and effective evaluation.

This test can help to reveal inner filter effects but not quenching. The test will work only if the Raman scattering peak is separated from the emission band of the petroleum hydrocarbons. If the Raman peak is superimposed on the slope of any other emission peak, the test is not conclusive.

Use 270 nm as the excitation wavelength. Fill the cell with pure hexane. Scan, mechanically or manually, the emission spectrum to find the maximum of the Raman scattering which should occur at 297 nm (to resolve the Rayleigh from the Raman scatter, narrow slits and high sensitivity settings must be used). Record the spectrum. Replace the hexane with the sample and scan in the same way to record the Raman scatter. It should be of the same intensity as that from the pure hexane. If it is significantly lower, an inner filter effect is present and the sample must be diluted and/or subject to an adsorption chromatography clean-up procedure.

Make a series of measurements of FI (310 nm/360 nm) of the sample at different dilutions. Plot FI vs the diluted concentrations. If a straight line is obtained there is no inner effect and any of the measurements can be used for quantification. If the relationship is not linear, dilute the sample until a linear relationship is obtained and use the FI at these dilutions for the quantification. For routine purposes, a single dilution can be used provided the results agree within 20%.

Measure the FI of the sample, then dilute it with an equal volume of a standard of chrysene or oil solution that has the same FI as the sample. Then measure the FI of the sample plus standard mixture. The FI of the mixture should be equal to:

$$\text{FI of mixture} = (\text{FI of standard})/2 + (\text{FI of sample})/2$$

If the FI of the mixture is less than that predicted by more than 20%, then significant inner filter effects exist and the FI of the sample must be multiplied by this correction factor for the quantification.

Note: The synchronous excitation/emission scan is much more selective to aromatic hydrocarbons by showing less interference from biogenic pigments. It is feasible to quantify by constructing calibration curves based on synchronous scans. At the time of revision of this manual, the U.S. Coast Guard Oil Spills Laboratory is experimenting to determine optimum wavelength differences.

8.5.3 High Performance Liquid Chromatography (HPLC)

This method is used to determine the concentration of certain polynuclear aromatic hydrocarbons (PAH) in ground water and wastes. Specifically this method is used to detect the following substances:

Analyte	CAS#
Acenaphthene	83-32-9
Acenaphthylene	
Anthracene	120-12-7
Benzo(a)anthracene	56-55-3
Benzo(a)pyrene	50-32-8
Benzo(b)fluoranthene	205-99-2
Benzo(ghi)perylene	191-24-2
Benzo(k)fluoranthene	207-08-9
Chrysene	218-01-9
Dibenzo(a,h)anthracene	53-70-3
Fluoranthene	206-44-0
Fluorene	86-73-7
Indeno(1,2,3-cd)pyrene	193-39-5
Naphthalene	91-20-3
Phenanthrene	85-01-8
Pyrene	129-00-0

Use of this method presupposes a high expectation of finding the specific compounds of interest. If the user is attempting to screen samples for any or all of the compounds listed above, the analyst must develop independent protocols for the verification of identity. The method detection limits for each compound in reagent water are listed in Table 8.9. Table 8.10 lists the practical quantitation limit for other matrices. The sensitivity of this method usually depends on the level of interferences rather than instrumental limitations. The limits of detection listed in Table 8.9 for the liquid chromatographic approach represent sensitivities that can be achieved in the absence of interferences. When interferences are present, sensitivity is decreased.

Prior to the use of this method, appropriate sample extraction techniques must be used (see Section 8.6.1). A 5- to 25-µL aliquot of the extract is injected into an HPLC, and compounds in the effluent are detected by ultraviolet (UV) and fluorescence detectors. If interferences prevent proper detection of the analytes of interest, the method may also be performed on extracts that have undergone cleanup using alumina column cleanup (see Section 8.5.1).

The chromatographic conditions described allow for a unique resolution of the specific PAH compounds covered by this method. Other PAH compounds in addition to matrix artifacts, may interfere.

"Apparatus, Materials, and Reagents - HPLC"

- **Kuderna-Danish (K-D) apparatus**

  Concentrator tube: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Ground-glass stopper is used to prevent evaporation of extracts.

  Evaporation flask: 500-mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with springs.

  Snyder column: Three-ball macro (Kontes K-503000-0121 or equivalent).

  Snyder column: Two-ball micro (Kontes K-569001-0219 or equivalent)

  Boiling chips: Solvent extracted, approximately 10/40 mesh (silicon carbide or equivalent).

  Water bath: Heated, with concentric ring cover, capable of temperature control (±5ºC). The bath should be used in a hood.

  Syringe: 5-mL.

  High pressure syringes.

- **HPLC apparatus:**

  Gradient pumping system: Constant flow.

  Reverse phase column: HC-ODS Sil-X, 5-micron particle size diameter, in a 250-mm x 2.6-mm I.D. stainless steel column (Perkin Elmer No. 089-0716 or equivalent).

  Detectors: Fluorescence and/or UV detectors may be used.

  Fluorescence detector: For excitation at 280-nm and emission greater than 389-nm cutoff (Corning 3-75 or equivalent). Fluorometers should have dispersive optics for excitation and can utilize either filter or dispersive optics at the emission detector.

  UV detector: 254-nm, coupled to the fluorescence detector.

  Strip-chart recorder: compatible with detectors. A data system for measuring peak areas and retention times is recommended.

  Volumetric flasks: 10-, 50-, and 100-mL.

  Reagent water: Reagent water is defined as water in which an interferent is not observed at the method detection limit of the compounds of interest.

  Acetonitrile: HPLC quality, distilled in glass.

  Stock standard solutions: Prepare stock standard solutions at a concentration of 1.00 µg/µL by dissolving 0.0100 g of assayed reference material in acetonitrile and diluting to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source. Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4ºC and protect from light. Stock standards should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Stock standard solutions must be replaced after one year, or sooner if comparison with check standards indicates a problem.

  Calibration standards: Calibration standards at a minimum of five concentration levels should be prepared through dilution of the stock standards with acetonitrile. One of the concentration levels should be at a concentration near, but above, the method detection limit. The remaining concentration levels should correspond to the expected range of concentrations found in real samples or should define the working range of the HPLC. Calibration

standards must be replaced after six months, or sooner if comparison with check standards indicates a problem.

Internal standards (if internal standard calibration is used): To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples. Prepare calibration standards at a minimum of five concentration levels for each analyte as described in above. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with acetonitrile.

Surrogate standards: The analyst should monitor the performance of the extraction, cleanup (if necessary), and analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard, and reagent water blank with one or two surrogates (e.g., decafluorobiphenyl or other PAHs not expected to be present in the sample) recommended to encompass the range of the temperature program used in this method. Deuterated analogs of analytes should not be used as surrogates for HPLC analysis due to coelution problems.

Extracts must be stored under refrigeration and must be analyzed within 40 days of extraction.

Prior to HPLC analysis, the extraction solvent must be exchanged to acetonitrile. The exchange is performed during the K-D procedures listed in all of the extraction methods. The exchange is performed as follows.

Following K-D of the methylene chloride extract to 1 mL using the macro-Snyder column, allow the apparatus to cool and drain for at least 10 min. Increase the temperature of the hot water bath to 95-100ºC. Momentarily remove the Snyder column, add 4 mL of acetonitrile, a new boiling chip, and attach a two-ball micro-Snyder column. Concentrate the extract using 1 mL of acetonitrile to prewet the Snyder column. Place the K-D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature, as required, to complete concentration in 15-20 min. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min. When the apparatus is cool, remove the micro-Snyder column and rinse its lower joint into the concentrator tube with about 0.2 mL of acetonitrile. A 5-mL syringe is recommended for this operation. Adjust the extract volume to 1.0 mL. Stopper the concentrator tube and store refrigerated at 4ºC, if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a Teflon-sealed screw-cap vial. Proceed with HPLC analysis if further cleanup is not required.

Using the column described, isocratically elute for 5 min using acetonitrile/water (4:6)(v/v), then linear gradient elution to 100% acetonitrile over 25 min at 0.5 mL/min flow rate. If columns having other internal diameters are used, the flow rate should be adjusted to maintain a linear velocity of 2 mm/sec.

Use Table 8.9 and especially Table 8.10 for guidance on selecting the lowest point on the calibration curve. Assemble the necessary HPLC apparatus and establish operating parameters equivalent to those indicated in above. By injecting calibration standards, establish the sensitivity limit of the detectors and the linear range of the analytical systems for each compound. Before using any cleanup procedure, the analyst should process a series of calibration standards through the procedure to confirm elution patterns and the absence of interferences from the reagents.

The estimated retention times of PAHs determinable by this method are summarized in Table 8.9. An example chromatogram is shown in Figure 8.1. If internal standard calibration is to be performed, add 10 µL of internal standard to the sample prior to injection. Inject 2-5 µL of the sample extract with a high-pressure syringe or sample injection loop. Record the volume injected to the nearest 0.1 µL, and the resulting peak size, in area units or peak heights. Re-equilibrate the HPLC column at the initial gradient conditions for at least 10 min between injections. Using either the internal or external calibration procedure, determine the identity and quantity of each component peak in the sample chromatogram which corresponds to the compounds used for calibration purposes. If the peak area exceeds the linear range of the system, dilute the extract and analyze. If the peak area measurement is prevented by the presence of interferences, further cleanup is required.

The quality control check sample concentrate should contain each analyte at the following concentrations in acetonitrile: naphthalene, 100 µg/mL; acenaphthylene, 100 µg/mL; acenaphthene, 100 µg/mL; fluorene, 100 µg/mL; phenanthrene, 100 µg/mL; anthracene, 100 µg/mL; benzo(k)fluoranthene, 5 µg/mL; and any other PA at 10 µg/mL.

The calibration and QC acceptance criteria for this method are summarized in Table 8.11. Method accuracy and precision as functions of concentration for the analytes of interest is summarized in Table 8.12. The contents of both Tables should be used to evaluate a laboratory's ability to perform and generate acceptable data by this method.

Calculate surrogate standard recovery on all samples, blanks, and spikes. Determine if the recovery is within limits.

If recovery is not within limits, the following procedures are required.

- Check to be sure there are no errors in calculations, surrogate solutions and internal standards. Also, check instrument performance.
- Recalculate the data and/or reanalyze the extract if any of the above checks reveal a problem.
- Reextract and reanalyze the sample if none of the above are a problem or flag the data as “estimated concentration.”

PERFORMANCE

The method was tested by 16 laboratories using reagent water, drinking water, surface water, and three industrial wastewaters spiked at six concentrations over the range 0.1 to 425 µg/L. Single operator precision, overall precision, and method accuracy were found to be directly related to the concentration of the analyte and essentially independent of the sample matrix. Linear equations to describe these relationships are presented in Tables 7-12.

Table 8.9. High Performance Liquid Chromatography of PAH(s)[a].

Compound	Retention Time (min)	Column Capacity Factor (K')	Method Detection Limit (mq/L) UV Fluorescence
Naphthalene	16.6	12.2	1.8
Acenaphthylene	18.5	13.7	2.3
Acenaphthene	20.5	15.2	1.8
Fluorene	21.2	15.8	0.21
Phenanthrene	22.1	16.6	0.65
Anthracene	23.4	17.6	0.64
Fluoranthrene	24.5	18.5	0.21
Pyrene	25.4	19.1	0.27
Benzo(a)anthracene	28.5	21.6	0.013
Chrysene	29.3	22.2	0.15
Benzo(b)fluoranthene	31.6	24.0	0.018
Benzo(k)fluoranthene	32.9	25.1	0.017
Benzo(a)pyrene	33.9	25.9	0.023
Dibenzo(a,h)anthracene	35.7	27.4	0.030
Benzo(ghi)perylene	36.3	27.8	0.076
Indeno(1,2,3-cd)pyrene	37.4	28.7	0.043

[a]HPLC conditions: Reverse phase HC-ODS Sil-X, 5 micron particle size, in a 250-mm x 2.6-mm I.D. stainless steel column. Isocratic elution for 5 min using acetonitrile/water (4:6)(v/v), then linear gradient elution to 100% acetonitrile over 25 min at 0.5 mL/min flow rate. If columns having other internal diameters are used, the flow rate should be adjusted to maintain a linear velocity of 2 mm/sec.

Table 8.10. Determination of Practical Quantitation Limits (PQL) for Various Matrices[a].

Matrix	Factor[b]
Ground water	10
Low-level soil by sonication with GPC cleanup	670
High-level soil and sludges by sonication	10,000
Non-water miscible waste	100,000

[a] Sample PQLs are highly matrix-dependent. The PQLs listed herein are provided for guidance and may not always be achievable.

[b] PQL = Method Detection Limit X [Factor]. For nonaqueous samples, the factor is on a wet-weight basis.

Table 8.11. QC Acceptance Criteria.

Parameter	Test Conc. (µg/L)	Limit for s (µg/L)	Range for x (µg/L)	Range P,P(s) (µg/L) (%)
Acenaphthene	100	40.3	D-105.7	D-124
Acenaphthylene	100	45.1	22.1-112.1	D-139
Anthracene	100	28.7	11.2-112.3	D-126
Benzo(a)anthracene	10	4.0	3.1-11.6	12-135
Benzo(a)pyrene	10	4.0	0.2-11.0	D-128
Benzo(b)fluoranthene	10	3.1	1.8-13.8	6-150
Benzo(ghf)perylene	10	2.3	D-10.7	D-116
Benzo(k)fluoranthene	5	2.5	D-7.0	D-159
Chrysene	10	4.2	D-17.5	D-199
Dibenzo(a,h)anthracene	10	2.0	0.3-10.0	D-110
Fluoranthene	10	3.0	2.7-11.1	14-128
Fluorene	100	43.0	D-119	D-142
Indeno(1,2,3-cd)pyrene	10	3.0	1.2-10.0	D-116
Naphthalene	100	40.7	21.5-100.0	D-122
Phenanthrene	100	37.7	8.4-133.7	D-155
Pyrene	10	3.4	1.4-12.1	D-140

s = Standard deviation of four recovery measurements, in µg/L.
x = Average recovery for four recovery measurements, in µg/L.
P, P(s) = Percent recovery measured.
D = Detected; result must be greater than zero.

Table 8.12. Method accuracy and Precision as Functions of Concentration[a].

Parameter	Accuracy, as recovery, x' (µg/L)	Single analyst precision s(r)' (µg/L)	Overall precision S' (µg/L)
Acenaphthene	0.52C+-0.54	0.39x+0.76	0.53x+1.32
Acenaphthylene	0.69C-1.89	0.36x+0.29	0.42x+0.52
Anthracene	0.63C-1.26	0.23x+1.16	0.41x+0.45
Benzo(a)anthracene	0.73+0.05	0.28x+0.04	0.34x+0.02
Benzo(a)pyrene	0.56C+0.01	0.38x-0.01	053x-0.01
Benzo(b)fluoranthene	0.78C_0.01	0.21x+0.01	0.38x-0.00
Benzo(ghf)perylene	0.44C+0.30	0.25x+0.04	0.58x+0.10
Benzo(k)fluoranthene	0.59C+0.00	0.44x-0.00	0.69x+0.10
Chrysene	0.77C-0.18	0.32x-0.18	0.66x-0.22
Dibenzo(a,h)anthracene	0.41C-0.11	0.24x+0.02	0.452+0.03
Fluoranthene	0.68C+0.07	0.22x+0.06	0.32x+0.03
Fluorene	0.56C-0.52	0.44x-1.12	0.63x-0.65
Indeno(1,2,3-cd)pyrene	0.54C+0.06	0.29x+0.02	0.42x+0.01
Naphthalene	0.57C-0.70	0.39x-0.18	0.41x+0.74
Phenanthrene	0.72C-0.95	0.29x+0.05	0.47xl-0.25
Pyrene	0.69C-0.12	0.25x+0.14	0.42x-0.00

x' = Expected recovery for one or more measurements of a sample containing a concentration of C, in µg/L.
s(r)' = Expected single analyst standard deviation of measurements at an average concentration of x, in µg/L.
S' = Expected interlaboratory standard deviation of measurements at an average concentration found of x, in µg/L.
C = True value for the concentration, in µg/L.
x = Average recovery found for measurements of samples containing a concentration of C, in µg/L.

[a]Criteria from 40 CFR Part 136 for Method 610. These criteria are based directly upon the method's performance. Where necessary, the limits for recovery have been broadened to assure applicability of the limits.

This method has been tested for linearity of spike recovery from reagent water and has been demonstrated to be applicable over the concentration range from 8 x MDL to 800 x MDL with the following exception: benzo(ghi)perylene recovery at 80 x and 800 x MDL were low (35% and 45%, respectively). The accuracy and precision obtained will be determined by the sample matrix, sample-preparation technique, and calibration procedures used.

8.5.4 Gas Chromatography/Mass Spectrometry (GC/MS)

This method outlines the procedures used for the quantitative determination of polynuclear aromatic hydrocarbons (PAH) in sample extracts using gas chromatography/mass spectrometry (GC-MS). The instrumental procedures described in this document are applicable to the quantitative analysis of extracts obtained from water, sediment, soil, tissue, and other sample matrices after appropriate extraction and purification (Section 8.5.1). The PAHs target compounds determined by this method and the surrogate used for quantitation (reference surrogate) are listed in Table 8.13. The analytical method detection limit (MDL) is determined on an annual basis. MDLs for the multi-analyte groups are estimated as twice the MDL of the parent compound.

The quality control requirements for quantitative analysis are summarized in Table 8.14 with details provided in the following sections.

The mass spectrometer performance is checked daily using PFTBA according to manufacturer's tuning procedures. These procedures include the checking of peak widths, mass axis calibration, and relative abundances of masses 69, 219, and 502 against manufacturer's recommended criteria. Isotope abundances are also checked according to the manufacturer's criteria. Total cycle time (dwell times plus switching times) for all ions within a single descriptor must be one second or less.

Qualitative identification of target compounds is based on a comparison of the retention times of the target compounds in the calibration curve with the retention time of the target compounds found in the sample extract. The retention time of the compound in the extract should be within ±4 seconds of the average retention time of the authentic compounds in the calibration standard. The ion current responses for ions used for quantitation and confirmation purposes must simultaneously reach their maxima (±2 seconds). The ion current responses for the quantitation and confirmation ions used for the labeled standards must reach maximum simultaneously (±2 seconds).

The extracted ion current profiles of the quantitation ion (primary m/z) and the confirmation ion (secondary ion) for each target compound must meet the following QC acceptance criteria**.** The characteristic masses of each target compound must maximize in the same scan or within one scan of each other. The retention time of target compounds in the sample must fall within ±4 seconds of the retention time for the authentic compound in the calibration standards. The relative peak heights of the primary ion compared to the confirmation (or secondary) ion mass for a target compound should fall within ±30 percent of the relative intensities of these masses in a mass spectrum (Table 8.15) obtained from a reference standard of

Table 8.13. Polynuclear Aromatic Hydrocarbons of Interest.

Compounds	I.S. Reference	Surrogate Reference	Compounds	I.S. Reference	Reference Surrogate
Naphthalene	A	1	Fluoranthene	B	3
C_1-Naphthalenes	A	1	C_1-Fluoranthenes[a]	B	3
C_2-Naphthalenes	A	2			
C_3-Naphthalenes	A	2	Pyrene	B	3
C_4-Naphthalenes[a]	A	2	C_1-Pyrene	B	3
Biphenyl	A	2	Benzo[a]anthracene	B	4
Acenaphthylene	A	2			
			Chrysene	B	4
Acenaphthene	A	2	C_1-Chrysene[a]	B	4
			C_2-Chrysene[a]	B	4
Fluorene	A	2	C_3-Chrysene[a]	B	4
C_1-Fluorenes[a]	A	2	C_4-Chrysene[a]	B	4
C_2-Fluorenes[a]	A	2			
C_3-Fluorenes[a]	A	2	Benzo[b]fluoranthene Benzo[k]fluoranthene } -sum	B	4
Dibenzothiophene	A	3	Benzo[e]pyrene	B	4
C_1-Dibenzothiophenes[a]	A	3	Benzo[a]pyrene	B	4
C_2-Dibenzothiophenes[a]	A	3	Perylene	B	5
C_3-Dibenzothiophenes[a]	A	3	Indeno[1,2,3-c,d]pyrene	B	4
			Dibenzo[a,h]anthracene	B	4
Phenanthrene	A	3	Benzo[g,h,i]perylene	B	4
C_1-Phenanthrenes	A	3			
C_2-Phenanthrenes[a]	A	3	<u>Specific Isomers</u>		
C_3-Phenanthrenes[a]	A	3			
C_4-Phenanthrenes[a]	A	3	1-methylnaphthalene	B	1
			2-methylnaphthalene	B	1
Anthracene	A	3	2,6-dimethylnaphthalene	B	2
C_1-Anthracenes[a]	A	3	2,3,5-trimethylnapthalene	B	2
C_2-Anthracenes[a]	A	3	1-methylphenanthrene	B	3
C_3-Anthracenes[a]	A	3			
C_4-Anthracenes[a]	A	3	<u>Surrogates</u>		
<u>Internal Standards</u>			Naphthalene-d_8	(1)	
			Acenaphthene-d_{10}	(2)	
Fluorene-d_{10}	(A)		Phenanthrene-d_{10}	(3)	
Benzo (a) pyrene-d_{12}	(B)		Chrysene-d_{12}	(4)	
			Perylene-d_{12}	(5)	

[a]Alkylated homologues not included in the calibration solution.

NOTE: Alkylated phenanthrenes and anthracenes, and alkylated fluoranthenes and pyrenes are quantified together as total alkylated (Cx) phenanthrene/anthracenes and total alkylated (Cx) fluoranthenes/pyrenes. Only the parent compounds and specific isomers are reported as individual compounds.

Table 8.14. Summary of QC Requirements for Quantitative Analysis.

Element	Control Limit Criteria	Frequency
- Instrument Calibration	Minimum of 5 standards; correlation coefficient of $\geq$ 0.99 or % RSD within ± 15% for all target compounds.	Initial and after any failures of continuing calibrations.
- Instrument Blank	Instrument free of interfering contamination or perform necessary maintenance.	Prior to analysis of all analytical batches.
- Reference Oil Solution	Analytes within ± 25% or average of lab certified concentration with no analytes > 35% or recalibrate.	Prior to analysis of all analytical batches.
- Continuing Calibration Verification (CCV)	Percent difference for all response factors within ± 15% or average of initial calibration; no single analyte greater than 25% or recalibrate and reanalyze back to last passing CCV.	After daily MS tune; once every 12 hours during the analytical sequence and at end of analytical sequence.
- Surrogate Recovery	Recovery of 40 to 120% for all surrogates. See Section 3.5.6 for corrective actions.	All samples.
- Method Blank	No analytes > 3x MDL. See Section 3.5.1 for exceptions to need for re-extraction.	One per QC batch.
- Duplicates (if applicable)	RPD $\leq$ 25% for all analytes > 10x MDL. See Section 3.5.4 for corrective action.	One per QC batch.
- Matrix Spike, Matrix Spike Duplicate (if applicable)	% recovery within 40 to 120%. RPD for the spike recoveries should be $\leq$ 25% for all analytes. See Sections 3.5.3 for corrective actions.	One per QC batch.
- Standard Reference Material (if applicable)	Recovery of 80% of certified or non-certified compounds within 30% of certified range for those analytes > 10x MDL. See Section 3.5.5 for corrective action.	One per QC batch.

Table 8.15. Parameters for Target Analytes.

Compound	Quantitation Ion	Confirmation Ions	% Relative Abudnance of Confirmation Ions
d_8-Naphthalene	136	134	15
Naphthalene	128	127	15
C_1-Naphthalenes (including isomers)	142	141	80
C_2-Naphthalenes	156	141	NA
C_3-Naphthalenes	170	155	NA
C_4-Naphthalenes	184	169,141	NA
d_{10}-Acenaphthene	164	162	95
Acenaphthylene	152	153	15
Biphenyl	154	152	30
Acenaphthene	154	153	98
d_{10}-Fluorene	176	174	85
Fluorene	166	165	95
C_1Fluorenes	180	165	NA
C_2-Fluorenes	194	179	NA
C_3-Fluorenes	208	193	NA
d_{10}-Phenanthrene	188	184	15
Phenanthrene	178	176	20
Anthracene	178	176	20
C_1-Phenanthrenes/anthracenes	192	191	NA
C_2-Phenanthrenes/anthracenes	206	191	NA
C_3-Phenanthrenes/anthracenes	220	205	NA
C_4-Phenanthrenes/anthracenes	234	219,191	NA
Dibenzothiophene	184	152,139	15
C_1-Dibenzothiophenes	198	184,197	NA
C_2-Dibenzothiophenes	212	197	NA
C_3-Dibenzothiophenes	226	211	NA
Fluoranthene	202	101	15
d_{12}-Chrysene	240	236	30
Pyrene	202	101	15
C_1-Fluoranthenes/pyrenes	216	215	NA
Benzo [a] anthracene	228	226	20
Chrysene	228	226	30
C_1-Chrysenes	242	241	NA
C_2-Chrysenes	256	241	NA
C_3-Chrysenes	270	255	NA
C_4-Chrysenes	284	269,241	NA
d_{12}-Benz (a)pyrene	264	260	20
Benzo [b] fluoranthene	252	253,125	30, 10
Benzo [k] fluoranthene	252	253, 125	30, 10
Benzo (e) pyrene	252	253	30
Perylene	252	253	20
d_{12}-Perylene	264	260	22
Benzo [a] pyrene	252	253, 125	30, 10
Indeno[1,2,3-c,d]pyrene	276	277, 138	25,30
Dibenzo [a,h] anthracene	278	279, 139	25,20
Benzo [g,h,i]pery1ene	276	277, 138	25,20

NA = Not Applicable

that target compound. A compound that does not meet secondary ion confirmation criteria may still be determined to be present in a sample after close inspection of the data by the mass spectroscopist. Supportive data includes the presence of the secondary ion having a ratio greater than ±30 percent of the primary ion which may be caused by an interference with the secondary ion. The data not meeting these criteria are reported but appropriately qualified.

Prior to all analytical sequences, a reference oil solution (~800 ng/L) is analyzed. This analysis is used to define the retention time windows for the multiple analyte groups. The patterns within the retention time window for the sample extract are compared to the pattern within the same window of the reference oil solution. The magnitude of the individual peaks may not be consistent but the location of the peaks within the window should be similar between the two analyses.

A five-point calibration curve based on response factors is established to demonstrate the linearity of the detector. The recommended standard concentrations are approximately 20, 100, 250, 500, and 1000 ng/mL. The QC acceptance criteria for linearity of the initial calibration curve requires that the percent relative standard deviation (RSD) of the response factors for each compound in the five calibration standards must be less than or equal to fifteen percent ($\leq$15%). If these RSD criteria are exceeded, the linearity of the initial calibration curve can be determined to be acceptable if the correlation coefficient (r) determined using linear least squares regression analyses is greater than or equal to 0.99. The instrument software generates and prints out both types of calibration data for all initial calibration determinations, which are maintained in calibration files with the raw data for that laboratory area. Calibration verification must be performed at the beginning of each analytical sequence. A calibration verification is also required at the end of a 12 hour shift or at the end of the analytical sequence, whichever is more frequent. QC acceptance criteria are based upon daily response factors for each compound which are compared to the mean response factors for the initial calibration curve. If the average daily response factors for all analytes is within ±15% of the calibration value, analyses may proceed. If, for any single analyte, the daily response factor exceeds ±25 percent of calibration value, the five-point calibration is repeated before analysis continues and all samples are re-analyzed back to last passing CCV.

The acceptance criteria for QC samples are evaluated within an analytical group. Therefore, failure of one QC sample type does not necessarily cause the entire analytical batch to fail. A method blank is used to demonstrate freedom from contamination in the analytical procedure, and is required for each set of 20 or fewer samples. If any of the target compounds are found in the blank at greater than 3x the MDL, re-extraction of the entire set may be required as specified in the following subsections. If any of the target compounds are found in the blank at greater than 3x the MDL, but are not detected in the analytical samples above the MDL, the analytical data can be reported and must be flagged, but no further action is required. When target compounds are present in the method blank and in the analytical samples at concentrations above 3x the MDL and the concentration in a sample is 10x that found in the blank, the blank must be flagged but sample data are reportable and are not flagged. When target compounds are present in the method blank and in the analytical samples at concentrations above 3x the MDL and the concentration in the sample is less than 10x that found in the blank, the sample should be re-extracted and re-analyzed. If no sample remains for re-extraction, the analytical data for those analytes in the blank and samples can be reported but must be flagged.

A laboratory blank spike (LBS) may be used to estimate analytical accuracy of the method if inadequate sample is available or if a complex matrix is present. It may be required with each set of 20 or fewer samples (Table 8.16). A laboratory blank spike duplicate (LBSD) is used to estimate both analytical accuracy and precision and may be required for each set of 20 or fewer samples. QC acceptance criteria for the target compound recoveries are that the recovery for each target compound falls between 40 and 120%. If the LBSD has been included, the recoveries determined from the LBS and LBSD should agree within an average RPD of $\leq$25%. If two or more of the target compounds are outside the QC acceptance criterion, corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the LBS and LBSD, re-extraction of the sample group, or instrument maintenance and/or recalibration. A laboratory blank spike may be used to demonstrate that the analytical system is in control when working with a difficult matrix or sample set.

A matrix spike (MS) sample is used to estimate analytical accuracy in the presence of a representative matrix and is normally required for each set of 20 or fewer samples A matrix spike duplicate (MSD) is used to estimate analytical accuracy and precision in the presence of a representative matrix and may be required for each set of 20 or fewer samples. QC acceptance criteria for target compound recoveries in the MS and the MSD is 40 to 120% of the spiked amount. In computing the QC acceptance criterion, only valid spikes will be used. In a valid spike, the amount of analyte added is at least as much as was originally present in the sample. If a MSD has been included with the analytical batch, the recoveries determined from the MS and MSD samples should agree within a RPD of $\leq$25%. The MS and MSD acceptance criterion are advisory. However, if two or more of the target compounds are outside the QC acceptance criterion, corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the MS and MSD, re-extraction of the sample/MS/MSD group, or instrument maintenance and/or recalibration.

A sample duplicate (DUP) is used to demonstrate sample homogeneity and analytical precision in the presence of a representative matrix and may be required with each set of 20 or fewer samples. QC acceptance criterion for analyte concentrations greater than ten times the MDL is a RPD of $\leq$25%. If the RPD is outside the QC acceptance criterion, corrective action may be indicated. Corrective action may include recalculation or reanalysis of the DUP and the original sample, instrument maintenance, recalibration, or re-extraction of the analytical batch.

A standard reference material (SRM) is used to demonstrate analytical accuracy and may be required with each set of 20 or fewer samples. When requested, a standard reference material is extracted and analyzed with each batch of samples. Acceptable concentrations are defined as the range of the documented (certified or reference) concentration plus or minus the 95% confidence limits in the certification. The QC acceptance criterion are that 80% of the data for measured concentrations should be within ±30% of the range of the acceptable concentration for each target compound (either certified or non-certified) with concentrations greater than 10 times the MDL. Corrective action may include recalibration or re-analysis of the SRM, instrument maintenance, re-calibration, or re-extraction of the analytical batch.

All samples are spiked with the appropriate surrogate spiking solution to determine the concentration of target compounds and to monitor method performance. QC acceptance criteria

Table 8.16. PAH Matrix Spike Compounds in CH_2Cl_2.

Compound	Spiking Solution Concentration (µg/mL)
Naphthalene	1.030 ± 0.10
1-Methylnaphthalene	1.24 ± 0.5
2-Methylnaphthalene	1.18 ± 0.04
Biphenyl	1.046 ± 0.04
2,6-Dimethylnaphthalene	1.08 ± 0.4
Acenaphthylene	1.040 ± 0.07
Acenaphthene	1.089 ± 0.15
2,3,5-Trimethylnaphthalene	0.99 ± 0.4
Fluorene	1.087 ± 0.08
Dibenzothiophene	~1.000
Phenanthrene	1.048 ± 0.07
Anthracene	1.169 ± 0.06
1-Methylphenanthrene	1.04 ± 0.3
Fluoranthene	0.884 ± 0.06
Pyrene	0.881 ± 0.08
Benz[a]anthracene	7.85 ± 0.05
Chrysene	1.050 ± 0.06
Benzo[b]fluoranthene	0.785 ± 0.05
Benzo[k]fluoranthene	0.833 ± 0.12
Benzo[e]pyrene	0.840 ± 0.04
Benzo[a]pyrene	1.014 ± 0.09
Perylene	1.065 ± 0.06
Indeno[1,2,3-cd]pyrene	0.940 ± 0.07
Dibenz[a,h]anthracene	0.774 ± 0.18
Benzo[ghi]perylene	0.790 ± 0.13

for surrogate compound recovery is 40 to 120% for all except d_{12}-perylene. The recovery of this surrogate is advisory only. If surrogate recovery fails the QC acceptance criteria, the following corrective action will be taken. The calculations are checked to ensure that there are no errors. The internal standard and surrogate solutions are checked for degradation, contamination, etc., and the instrument performance is checked. If the surrogate recovery is outside the control limits, the secondary ion may be used to check the quantitation of the surrogate. If the secondary ion is within the control limits, this recovery can be used and the data are appropriately annotated. If the upper control limit is exceeded for only one surrogate, and the instrument calibration and other surrogate standard concentrations are in control, it is concluded that an interference specific to the surrogate was present that resulted in high recovery and that this interference does not affect the quantitation of other target compounds. The presence of this type of interference is confirmed by evaluation of chromatographic peak shapes. To correct for the underestimation of the analyte concentration based on this surrogate, the target compounds will be quantified using the surrogate that is chromatographically closest to the surrogate exhibiting interference. If the surrogate cannot be measured because of the amount and nature of interferants in the sample, the target compounds based on that surrogate will be quantified based on the closest surrogate. The surrogate recovery is appropriately qualified. If the native concentration of hydrocarbons are high and require dilution for acceptable chromatographic separations, a dilution is made. A known aliquot of the extract is removed. One hundred (100)

µL of surrogate are added and the volume brought to 1.0 mL. The appropriate changes are made in the dilution factor within the quantitation software and the sample is re-analyzed. There is no concentration correction based on the surrogate recovery for dilution results. The surrogate recoveries are not reported but qualified with a "D" to denote the dilution. The MDL must also be adjusted to account for any dilutions. The extract is reanalyzed if the steps above fail to reveal a problem. If re-analysis of the extract yields surrogate recoveries within the stated limits, then the re-analysis data is reported. If re-analysis does not yield acceptable recoveries, the samples will be re-extracted. If re-analysis does not improve surrogate recovery, the data are reported and properly qualified.

Representative aliquots are injected into the capillary column of the gas chromatograph using the following conditions:

Injector Temp:	300°C
Transfer Line Temp:	280°C
Initial Oven Temp:	60°C
Initial Hold Time:	0 min
Ramp Rate:	12°C
Final Temperature:	300°C
Final Hold Time:	18 min

The effluent from the GC capillary column is routed directly into the ion source of the mass spectrometer. The MS is operated in the selected ion monitoring (SIM) mode using appropriate windows to include the quantitation and confirmation masses for the PAHs listed in Table 8.15. For all compounds detected at a concentration above the MDL the confirmation ion is checked to confirm its presence. The analytical system includes a temperature programmable gas chromatograph (Hewlett-Packard 5890A, or equivalent). The injection port is designed for split or splitless injection and analyses are conducted in the splitless mode. A 30-m long x 0.32-mm I.D. fused silica capillary column with DB-5MS bonded phase (J&W Scientific or equivalent) is used. The autosampler is capable of making 1 to 4 µL injections. The mass spectrometer (HP 5970/72 MSD) operates at 70 eV electron energy in the electron impact ionization mode and is tuned to maximize the sensitivity of the instrument based on manufacturer specifications.

A surrogate solution is made by weighing appropriate amounts of pure compounds into a volumetric flask and diluting to volume with methylene chloride. Surrogates are added to the samples prior to extraction at a concentration of approximately 10x the MDL. If higher concentrations of hydrocarbons are anticipated, the surrogate concentration can be appropriately increased. The compounds in the surrogate solution are deuterated aromatics (Table 8.13). The concentration of the surrogate solution requires the addition of 100 µL to the extract, leading to a surrogate final concentration of 40 ng/mL in the final extract volume. All sample target compound concentrations are corrected for surrogate recoveries.

A solution containing two internal standards at 20 µg/mL is prepared based on weight from a certified standard. The stock solution is transferred to a volumetric flask and diluted to volume with methylene chloride. The internal standards are deuterated aromatics (Table 8.13). Sufficient internal standard solution is added to the extract just prior to instrumental analysis to give a final concentration of 40 ng/mL in the final extract volume.

A solution containing selected PAHs is used to fortify blank spikes and matrix spike samples. A certified solution is purchased from a commercial vendor and diluted with methylene chloride to the appropriate working concentration. Dibenzothiophene is weighed neat and added to the spiking solution to make a final concentration of about 1.0 µg/mL. The spiking solution is added to give a final concentration of approximately 10x the MDL. If higher concentrations of hydrocarbons are anticipated, the matrix spike can be appropriately increased.

Initial calibration is required before any sample is analyzed for PAHs. Initial calibration is also required if the analysis of a calibration verification standard does not meet the required criteria. Tune the instrument with PFTBA as described. Using the same GC and MS conditions that produced acceptable tune results, analyze a 2 µL portion of each of the five calibration solutions once, and demonstrate that the following conditions are met. The ratio of integrated ion currents for the quantitation and confirmation ions appearing in Table 8.15 must be within ±30% the indicated percent relative abundance established for each compound. The ratio of integrated ion currents for the quantitation and confirmation ions belonging to the surrogates and the internal standards must be within the ±30% of the relative abundance stipulated in Table 8.15. All ratios must be within the specified acceptance limits simultaneously in one run. Otherwise, corrective action is necessary.

For each injection, calculate the relative response factors (RRF) for target compounds relative to their appropriate quantitation standards according to Equation 1. The following formula is used to calculate the response factors (RRF) of target compounds relative to its reference surrogate in the calibration standards.

$$RRF = (A_s C_{su})/(A_{su} C_s) \qquad \text{Equation 1}$$

where:

A_s = Area of the quantitation ion for the target compound.
A_{su} = Area of the quantitation ion for the surrogate.
C_{su} = Concentration of the surrogate (ng/µL).
C_s = Concentration of the target compound to be measured (ng/µL).

The response factors for the multi-analyte groups are assumed to be that of the parent compound.

Calculate the mean response factors ($\overline{RRF}$s) for the five calibration solutions using Equation 2.

$$\overline{RRF} = \left(\frac{1}{5}\right) \sum_{j=1}^{5} RRF_j \qquad \text{Equation 2}$$

where:

j = the injection number or calibration solution number (j = 1 to 5).

Determine the respective percent relative standard deviation for each compound in the calibration standards (%RSD) by dividing the standard deviation by the mean response factor and multiplying the result by 100. Document that the initial calibration meets all of the acceptance criteria.

Continuing calibration verification must be performed at the beginning of an analytical sequence following successful MS tune except when following an initial calibration. A continuing calibration verification is also required at the end of a 12 hour analysis period or at the end of the analytical sequence, whichever is more frequent. Using the same GC and MS conditions that were used for the initial calibration, analyze a 2 µL portion of the 250 ng/mL calibration solution and evaluate it with the following QC acceptance criteria. The ratio of integrated ion currents for the quantitation and confirmation ions appearing in Table 8.15 must be within the ±30% of the relative abundance established for each target compound. The ratio of integrated ion currents of the quantitation and confirmation ions for the surrogate and internal standards must be within the ±30% of the relative abundance stipulated in Table 8.15. Note: All ratios must be within the specified acceptance limits simultaneously in one run. Otherwise, corrective action is necessary.

Calculate the daily response factor for the target compounds using Equation 1. These daily response factors for each compound are then compared to the mean response factors from the initial calibration curve. The percent difference is calculated using Equation 3:

$$\text{Percent Difference} = \frac{(\overline{\text{RRF}} - \text{RFC}) \text{ x } 100}{\overline{\text{RRF}}} \qquad \text{Equation 3}$$

where:

$\overline{\text{RRF}}$ = Mean response factor from initial calibration.

RFC = Response factor from current verification check standard. The QC acceptance criteria requires that the average daily response factors for all analytes must be within ±15% of the calibration value for the analyses to proceed. If, for any single analyte, the daily response factor exceeds ±25 percent of the calibration value, the five point calibration must be repeated prior to further analysis.

Tune the instrument with PFTBA as described. Inject 2 µL of methylene chloride as an instrument blank, and acquire SIM mass spectra data as described. Demonstrate and document that the analytical system is free from interfering contamination. Inject 2 µL of the Standard Oil Solution and acquire SIM mass spectral data as described. Demonstrate and document that the retention time windows have been established and the criteria are met. Inject 2 µL of the calibration verification standard (250 ng/mL) and acquire SIM mass spectral data as described. Demonstrate and document that the criteria are met. Inject 2 µL of the sample extract and acquire SIM mass spectral data under the same conditions that have been established to produce acceptable results.

For a gas chromatographic peak to be identified as a target compound, it must meet all of the criteria specified for single analyte compounds or for multiple analyte compounds.

For gas chromatographic peaks that have met all the qualitative identification criteria, calculate the concentration of the target compounds using Equation 4. Based on these response

factors, sample extract concentrations for each analyte are calculated using the following formula:

$$C = \frac{(A_S)(C_{SU})}{(A_{SU})(\overline{RRF})(Sa)} \qquad \text{Equation 4}$$

where:

C	=	Concentration in sample (ng/gram or ng/liter).
Sa	=	Sample amount (grams, liters).
A_S	=	Area of the quantitation ion for the target compound to be measured.
A_{SU}	=	Area of the quantitation ion for the surrogate.
C_{SU}	=	Amount of surrogate added to each extract (ng).
$\overline{RRF}$	=	Average response factor.

Calculate the percent recovery of the five surrogate quantitation standards in the sample extract using Equation 5.

$$\% \text{ recovery} = \frac{(A_{SU} \text{ x } C_{IS})}{(C_{SU} \text{ x } A_{IS} \text{ x } \overline{RRF}_{SU})} \qquad \text{Equation 5}$$

where:

A_{IS}	=	Area of the quantitation ion for the appropriate internal standard.
A_{SU}	=	Area of the quantitation ion for the surrogate.
C_{SU}	=	ng of deuterated surrogate added to the sample.
C_{IS}	=	ng of deuterated internal standard added to the sample extract.
$\overline{RRF}_{SU}$	=	Average response factor for the surrogate based on the internal standard from the initial calibration.

The laboratory will take corrective action whenever the recovery of any surrogates is less than 40% or greater than 120%. If the concentration in the final extract of any of the target compounds exceeds the upper method calibration limit, the sample extract must be diluted by an appropriate dilution factor and reanalyzed. Detection limits must also be adjusted to compensate for sample dilution. Calculate and report the Relative Percent Difference (RPD) between duplicate sample results. Calculate and report the % Recovery of target compounds in the Matrix Spike (MS), Matrix Spike Duplicate (MSD), and, if analyzed, in the Laboratory Blank Spike (LBS) samples.

Gas chromatograph maintenance consists of the following:

The syringe is cleaned by rinsing with appropriate solvent after each injection. A new injection port liner and septum are installed at the beginning of each new run sequence. A new injection port base plate is installed as needed. A length (30-50 cm) of the analytical column is removed as needed. This is necessary when there is significant tailing of the peak shapes. The tank of carrier gas (He) is replaced when the pressure falls below 500 psi. All instrument maintenance is recorded in the maintenance log for the specific instrument.

Mass spectrometry maintenance consists of the following:

The emission filament is replaced as necessary. The source assembly is cleaned and replaced as necessary. The PFTBA reservoir is refilled as necessary. The transfer line/re-entrant assembly is disassembled, cleaned or repaired and reassembled as necessary. The rotary pump oil is changed yearly, or more frequently if indicated. The diffusion pump oil is changed as necessary. All maintenance is recorded in the maintenance log for the specific instrument.

PERFORMANCE

Quality control samples are processed in an identical manner as actual samples. A method blank is run with every 20 samples, or with every sample set, whichever is more frequent. Blank levels should be no more than 3x method detection limit (MDL). If blank levels for any component are above 3x MDL, samples analyzed in that sample set should be re-extracted and reanalyzed. If insufficient sample is available for extraction, the data will be reported and appropriately qualified. Matrix spike/matrix spike duplicate (MS/MSD) samples are run with every 20 samples, or with every sample set, whichever is more frequent. The appropriate spiking level is 3 to 10x the MDL. Surrogate materials are spiked into every sample and QC sample. The appropriate spiking level is 3 to 10x the MDL.

STANDARD REFERENCE MATERIALS

Reference Materials: A sediment fortified with crude oil, when available, is analyzed for aliphatic and aromatic hydrocarbons. One reference material per batch (~20 samples) will be analyzed to establish control charts for these analyses. Other appropriate standard reference material from NIST may be substituted.

CHAPTER 9.
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Glossary of Terms

Accuracy—combination of bias and precision of an analytical procedure, which reflects the closeness of a measured value to a true value.

Agglomeration—A technique that combines powdered material to form larger, more soluble particles by intermingling in a humid atmosphere. [Meterol] The process in which particles grow by collision with and assimilation of cloud particles or other precipitation particles. Also known as coagulation. An indicriminantly formed cluster of particles.

Bias—consistent deviation of measured values from the true value, caused by systematic errors in a procedure.

Calibration check standard—standard used to determine the state of calibration of an instrument between periodic recalibrations.

Carcinogen—Any agent that incites development of a carcinoma or any other sort of malignancy.

Conductivity—The ratio of the electric current density to the electric field in a material.

Confidence coefficient—the probability, *%,* that a measurement result will lie within the confidence interval or between the confidence limits.

Confidence interval—set of possible values within which the true value will lie with a specified level of probability.

Confidence limit—one of the boundary values defining the confidence interval.

Data archival—A method for the safe storage and easy retrieval of all accumulated data, photographs, and videotapes.

Data dictionary—A catalog of all the attributes for a data set as well as the constraints placed on these attributes values depending on their definition. Also, the part of the database that contains information about files, records, and attributes as well as their data.

Data Screening Analysis—A quality control procedure designed to minimize error.

Detection limits—Various limits in increasing order.

Dissolved Oxygen—the volume of oxygen that is contained in a solution.

Duplicate—usually the smallest number of replicates (two) but specifically herein refers to duplicate samples. i.e., two samples taken at the same time from one location.

Instrumental detection limit (IDL)—the constituent concentration that produces a signal greater than five times the signal/noise ratio of the instrument. This is similar, in many respects, to "critical level" and "criterion of detection." The latter limit is stated as *1.645* times the *s* of blank analyses.

Internal standard—a pure compound added to a sample extract just before instrumental analysis to permit correction for inefficiencies.

Laboratory control standard—a standard, usually certified by an outside agency, used to measure the bias in a procedure. For certain constituents and matrices, use National Institute of Standards and Technology (NJST)* Standard Reference Materials when they are available.

Limit of quantitation (LOQ)—the constituent concentration *that* produces a signal sufficiently greater than the blank that it can be detected within specified limits by good laboratories during routine operating conditions.' Typically *it* is the concentration that produces a signal lOs above the reagent water blank signal.

Lower limit of detection (LLD)—the constituent concentration in reagent water that produces a signal ^{2}*(1.645)s* above the mean of blank analyses. This sets both Type I and Type II errors at 5%. Other names for this limit are "detection limit" and "limit of detection" (LOD).

Metadata—Data about the data. Index-type information pertaining to the entire data set, which includes the following: date, source, map projection, scale, resolution, accuracy, and reliability of the information as well as format and structure of the data set.

Method detection limit (MDL)—the constituent concentration that, when processed through the complete method, produces a signal with a 99% proability that it is different from the blank. For seven replicates of the sample, the mean must be 3.14s above the blank where *s* is the standard deviation of the seven replicates. The MDL will be larger than the LLD because of the few

replications and the sample processing steps and may vary with constituent and matrix.

pH—a term used to describe the hydrogen-ion activity of a system; it is equal to $- \log a_{H^+}$; here a_{H^+} is the activity of the hydrogen ion; in dilute solution, activity is equal to concentration and pH is defined as $- \log_{10} [H^+]$, where H^+ is the hydrogen ion concentration in moles per Liter; a solution of pH 0 to 7 is acid, pH of 7 is nuetral, pH of 7 to 14 is alkaline.

Photometer—An instrument used for making measurements of light or electromagnetic radiation in the visible range.

Planchet—A small metal container or sample holder; usually used to hold radioactive materials that are being checked for the degree of radioactivity in a proportional counter or scintillation detector.

Precision—measure of the degree of agreement among replicate analyses of a sample, usually expressed as the standard deviation.

Quality assessment—procedure for determining the quality of laboratory measurements by use of data from internal and external quality control measures.

Quality assurance—A series of planned or systematic actions required to provide adequate confidence that a product or service will satisfy given needs.

Quality control—set of measures within a sample analysis methodology to assure that the process is in control.

Random error—the deviation in any step in an analytical procedure that can be treated by standard statistical techniques.

Reagent—A substance, chemical, or solution used in the laboratory to detect, measure, or otherwise examine other substances, chemicals, or solutions; grades include ACS, reagent (for analytical reagents, CP (chemically pure), USP (U.S. Pharmacopeia standards), NF (National Formulary Standards, and purified technical (for industrial use). The compound that supplies the molecule, ion, free radical which is considered the attacking species in a chemical reaction.

Raster Data—A data structure for maps based on grid cells.

Replicate—repeated operation occurring within an analytical procedure. Two or more analyses for the same constituent in an extract of a single sample constitute replicate extract analyses.

Standard Reference Material—A reference material distributed and certified by an appropriate supplier.

Surrogate standard—a pure compound added to a sample in the laboratory just before processing so that the overall efficiency of a method can be determined.

Toxicity—1. The quality of being toxic. 2. The kind and amount of poison or toxin produced by a microorganism, or possessed by a chemical substance not of biological origin.

Type I error—also called alpha error, is the probability of deciding a constituent is present when it actually is absent.

Type II error—also called beta error, is the probability of not detecting a constituent when it actually is present.